Contrasting effect of high-starch and high-sugar diets on cattle

Andrea Francesio, DVM

Submitted in fulfilment of the requirement for the
Degree of Master of Veterinary Medicine

Scottish Centre for Production Animal Health and Food Safety
School of Veterinary Medicine
College of Medical, Veterinary & Life Sciences
University of Glasgow

January, 2020

Abstract

Subacute Ruminal Acidosis (**SARA**) is a metabolic pathology of high yielding dairy cows traditionally linked with daily bouts of low ruminal pH. Due to its mild clinical appearance, on field diagnosis of SARA is difficult and often the result of a "by exclusion" process. Several attempts have tried in the past to developed diagnostic sensor systems capable of correctly identifying the disease. Our trial investigated the systemic effect of two different sources of carbohydrates hypothesising that sugars and starches would have reflected differently on rumen milieu and, hence, on animal health. Secondly, we compared and discussed different statistical models and their effectiveness to detect acidotic disturbances. The last objective of the study was to explore the capability of a sensor developed to continually monitor reticuloruminal contraction, to diagnose SARA under experimental conditions. The study, designed as a 3 × 3 Latin square, lasted nine weeks and involved six adult cows. During the first two weeks of each experimental period cows were fed with a maintenance diet and in the last week of each experimental period animals were allocated to a control (**CON**), a high starch (**HSt**) and a high sugar diet (**HSu**). Reticular pH and motility were recorded throughout the study, blood and ruminal samples were taken the 1st, the 2nd and the last day of each challenge week. Cows' health was monitored daily for diarrhoea, inappetence, depression and ruminal tympany. Blood parameters were analysed with linear mixed effects models (**LME**). pH data were analysed using three different models. In the first one, numbers of minutes for which pH < 5.6 and pH < 5.8, were obtained for each cow for each day and, in the second one, randomly chosen samples subsets were compared against the same thresholds. In the last approach, data points were used to generate a list of predicted values for each animal according to a generalised additive model. For each data point, the absolute value of the residual was used for the subsequent analysis. Motility readings were described according to their amplitude and period. Both challenge diets decreased the inter-contraction period and HSt diet only increased contraction amplitude. HSu diet was more likely to be not eaten and both the challenge diets were more likely to cause diarrhoea compared with CON diet. HSt diet increased plasma concentration of Serum Amyloid A (**SAA**) and neutrophils, affected several inflammatory markers and VFA concentration while CON and HSu diets resulted in less significant changes. All the models tested, indicated HSu diets reflected more severely on rumen pH and no model resulted in a better prediction of inflammatory response. In conclusion this study indicated that similar values in ruminal pH can reflect differently on clinical and subclinical health parameters, suggesting that ruminal pH is a suboptimal marker for rumen health.

Table of Contents

Abstract 2
Table of Contents 3
List of Tables 5
List of Figures 6
Acknowledgement 7
Author's Declaration 8
Abbreviations 9
Chapter 1 Literature Review and Introduction 11
1.1 Rumen physiology 11
1.1.1 Rumen metabolism 11
1.1.2 Neural control of rumen motility 15
1.2 Ruminal acidotic disorder 20
1.2.1 Carbohydrates dysmetabolism and its consequences 20
1.2.2 Clinical definition of SARA and its criticisms 24
1.2.3 Prevalence of SARA across European dairy herds 26
1.2.4 Differential effects of dietary composition on rumen health 28
1.3 Continuous monitoring of cattle health 29
1.3.1 Indwelling sensors applied to SARA diagnosis 31
1.3.2 Use of indwelling motion bolus to measure rumen motility 33
1.4 Scope of the thesis 34
Chapter 2 Material and Methods 39
2.1 Study design 39
2.2 Sample collection and process 41
2.3 Feed, blood and ruminal samples analysis 42
2.3.1 Haematology 42
2.3.2 Biochemistry 43

2.3.3 Serum Amyloid A determination 43
2.3.4 Haptoglobin determination 44
2.3.5 Ethanol and Volatile Fatty Acids determination 44
2.3.6 Histamine determination 44
2.4 Continuous measurement of ruminal pH 45
2.5 Continuous measurement of ruminal motility 46
2.6 Statistical analysis 46
2.6.1 Health, haematological and biochemical parameters 46
2.6.2 Reticuloruminal pH and motility 46
Chapter 3 Results 48
3.1 Feed intake and health parameters 48
3.2 Haematology and biochemistry 49
3.3 Ruminal pH 54
3.4 Ruminal motility 62
Chapter 4 Discussion 67
4.1 Comparison of the systemic effect of the challenge diets 67
4.2 Efficacy of recent descriptive ruminal pH models applied to SARA diagnosis 70
4.3 Evaluation of the capability of newly developed sensor system to diagnose SARA under experimental condition 72
Conclusion 75
Bibliography 76

List of Tables

Table 1-1. Major components of rumen microbiome, their substrates and products synthesised (Van Soest, 1994) 14
Table 1-2. Prevalence of SARA across European dairy herds - cow level.....27
Table 1-3. Prevalence of SARA across European dairy herds - herd level 27
Table 2-1. Diet ingredients and nutrient composition of the three diets fed in the trial 40
Table 2-2 Coeffients of variation and lower detection limits of the assays used.....42
Table 3-1. Effects of treatment on the binary health indicators and feed consumption.....48
Table 3-2. Haematological analytes according to diet fed and time of sampling. 50
Table 3-3. Biochemical serological analytes according to diet fed and time of sampling...51
Table 3-4. Biochemical ruminal analytes according to diet fed and time of sampling.....53
Table 3-5. Time below pH tresholds according diets and sequence day 55
Table 3-6. Single point sampling simulation according to dietary treatment 56

List of Figures

Figure 1-1. Central modulation of reticuloruminal motility 19
Figure 1-2. Sensors' evaluation framework as described by Rutten et al. (2013) 37
Figure 1-3. Stage of development of dairy sensor systems.....37
Figure 2-1. Experimental feeding management.....40
Figure 2-2. Sampling time during the trial.....41
Figure 3-1. pH curves according to diet fed and hour of the day.....57
Figure 3-2. Time below threshold (pH < 5.6) according to diet fed and hour of the day 58
Figure 3-3. Time below threshold (pH < 5.8) according to diet fed and hour of the day 59
Figure 3-4. Time below pH tresholds according diets.....60
Figure 3-5. Distribution of mean absolute residuals by diet fed and hour of day.....61
Figure 3-6. Period and amplitude of the motility readings registerd 63
Figure 3-7. Daily variation of contractions' amplitude by hour of day and diet fed 64
Figure 3-8. Daily variation of contractions' frequency by hour of day and diet fed 65
Figure 3-9. Diets effect on reticulorumen motility 66

Acknowledgement

Before I may consider this very fascinating and demanding work completed, I feel obliged to thank a few people who, with their joined efforts, made it possible.
Firstly, I would like to thank Prof. Nicholas Jonsson for having been invaluable guide during the ideation, the development and the fulfilment of this research project. His extensive knowledge and pleasant personality make him an excellent point person for every young researcher.

A special thank is due to Lorenzo Viora who led me throughout the extension of my Combined 4-year Residency and Masters Programme in Bovine Health Management providing his crucial support when it was of the utmost importance.

Many other people cooperated with their personal expertise to the realisation of this research. Among others I am pleased to remember here Holly Ferguson and Nicola Brady for their help in the laboratory, Will Tulley for the precise design of the experimental diets, Peter Hastie, holder of the project licence and Matt Denwood for his contribution in the statistical analysis.

A heartfelt thank is due to my family, which, despite the distance, has always been of huge and prompt support.

The last thank is for my colleagues and friends of the Scottish Centre for Production Animal Health & Food Safety. Working with you has been a pleasure and a privilege and I am deeply grateful for the occasion I had to join this team. I wish you all the best!

Author's Declaration

I declare that, except where explicit reference is made to other people contribution, this dissertation is the result of my own work and is not been submitted for any other degree at the University of Glasgow or any other institution.

Name: Andrea Francesio

Signature:

Abbreviations

APP: acute phase proteins
AST: aspartate aminotransferase
BHB: β-hydroxybutyrate
CHO: carbohydrate
CON: control
CV: coefficients of variation
DMI: dry matter intake
Eh: redox potential
F:C: forage to concentrate ratio
GGT: gamma-glutamyl transferase
GLDH: glutamate dehydrogenase
Hp: Haptoglobin
HSt: high-starch
HSu: high-sugar
IL-1β: Interleukin-1β
IL-6: Interleukin-6
LBP: LPS-binding proteins
LME: linear mixed effect models
LPS: lipopolysaccharides
min/d: minutes/day
NEB: negative energy balance
NEFA: non-esterified fatty acids
NF-κB: nuclear factor-κB
NSC: non-structural carbohydrates
peNDF: physically effective neutral detergent fibre
SAA: Serum Amyloid-A
SARA: subacute ruminal acidosis
TLR: toll-like receptors
TMR: total mixed ration
TNF-α: Tumour Necrosis Factor-α
TP-1: Time Point-1
TP-2: Time Point-2
TP-7: Time Point-7

VFA: volatile fatty acids

Chapter 1 Literature Review and Introduction

1.1 Rumen physiology

1.1.1 Rumen metabolism

1.1.1.1 The ruminal milieu

The reticulorumen is the result of the evolutionary process, by which ruminants have attempted to maximise their ability to utilise cellulosic carbohydrates (**CHO**). It is an extremely diverse, complex and highly-regulated ecosystem in which bacteria, protozoa and fungi interact with different substrates and with each other, transforming raw substrates into energy and more complex products, such as volatile fatty acids (**VFA**), vitamins and proteins. Under physiological conditions, the rumen is an anaerobic environment, suitable for the growth of some facultative anaerobes microbes. Its temperature is usually between 38 and 41 °C, its osmolality is below 300 mOsm/L and reductions and hydrolysis are the main reactions that take place in that environment. Physiological ruminal pH is traditionally reported to be between 6.0 and 6.8 and it is the result of introduction, production and absorption of VFA and ammonia as well as the presence of bicarbonates and phosphates as buffering systems (Hoover & Miller, 1991; Owens et al., 1998).

1.1.1.2 Rumen microbiome

Bacteria are the more represented microbial population in the rumen ecosystem, they are present in a total concentration 10^9 – 10^{10} cells/gram of rumen content and they exist associated with feed particles as well as free in the rumen fluid and adherent to the mucosal epithelium (Matthews et al., 2019). Historically, they have been classified on the basis of the substrate they metabolise and the end products of their metabolism as cellulolytic, amylolytic and proteolytic. Microbes have also been classified as primary or secondary fermenters, according to whether they break down nutrients introduced with the feed or generated as a product of primary fermentation (Van Soest, 1994; Sjaastad, Hove, & Sand, 2003). However only about 15% of rumen bacteria can be cultured, so, in recent years, there has been a growing interest in molecular technologies. Genome sequencing, for example, has unlocked the discovery of several enzymes and the full genome sequence of some bacteria is now

publicly available (Morgavi et al., 2013). Although the potential of the –omics approaches is undeniable, many aspects of rumen metabolism and bacteria-host interactions have been elucidated in more than 60 years of research, since Hungate's masterpiece "The rumen and its microbes" was published in 1966. This has led to a core of knowledge that is unlikely to be revolutionised by new technologies which, nonetheless, will be essential to fully understand function and ecology of rumen microbiome and, possibly in the future, to adequately predict causes and effects of its shifting (Morgavi et al., 2013; Gruninger et al., 2019). A list of the major species of rumen bacteria, along with substrates metabolised and products formed, is shown in Table 1–1.

Protozoa are present in variable concentrations depending on the diet type. As a general rule, their number increases with the amount of starch fed, until the production of acid decreases ruminal pH to 5.5 or below (Hoover & Miller, 1991). However in a favourable environment, they can be as numerous as 10^5 – 10^6 cells/gram of rumen content and, because of their greater size, they can contribute up to 50% of total microbial mass (Baldwin & Allison, 1983; Hoover & Miller, 1991). Protozoa are directly involved in cellulolytic metabolism but their principal regulatory role in rumen metabolism might the engulfment of starch, protein and bacteria (Van Soest, 1994).

Anaerobic fungi are the smallest and the most recently recognised portion of the microbiome, they cooperate to the cellulolytic activity of the microbiome, through their high production of cellulases, hemicellulases, as well as increasing lignin metabolism efficiency by breaking lignified structures through their rhizoids (Hoover & Miller, 1991; Van Soest, 1994; Matthews et al., 2019).

1.1.1.3 Ruminal metabolic processes

As, previously stated, ruminants have evolved to optimise the digestion of cellulosic, or structural, carbohydrates. These are polymers of glucose, xylose, arabinose, and glucuronic acid, generally linked β 1 – 4 like cellulose and hemicellulose. Pectin, being a galacturonic acid polymer linked α 1 – 4, is an exception among structural carbohydrate, as its bond is more typical of water-soluble, or non-structural, carbohydrates (**NSC**). Structural carbohydrates are components of the plant cell wall and they cannot be metabolised by mammals' digestive enzymes (Allen, 1991). Other sources of carbohydrates are NSC, and

they include water-soluble mono-, di-, oligo- and polysaccharides. Among NSC starch is the most important fraction because its high energy density and its widespread use in cattle nutrition. Starches are a polymer of glucose units linked α 1 – 4 and branched as α 1 – 6 and, as such, they could be digested by mammals (Allen, 1991). However, despite their different chemical nature, carbohydrates are metabolised through common pathways.

The first step of carbohydrate metabolism is usually the hydrolysis of polymers to mono-, di- and oligo-saccharides. Hydrolysis happens in the intra- and extra-cellular space by bacterial endo- and exo-hydrolase. Products of primary fermentations are then fermented anaerobically through the glycolytic pathways providing two molecules of ATP, pyruvate and $NADH_2$ for each cycle. The fate of pyruvate and $NADH_2$ vary greatly depending upon the rumen milieu. Pyruvate can be converted into lactate, acetate, propionate or butyrate while $NADH_2$ can be oxidised by methanogenic or acetogenic bacteria with the formation of methane or acetate, CO_2 and H_2 respectively (Baldwin & Allison, 1983; Van Soest, 1994).

Lipids are scarcely metabolised in the reticulorumen, as rumen microbes do not use fatty acids as energy source (Emery & Herdt, 1991). The main ruminal reactions involving lipids are hydrogenation of unsaturated fatty acids and hydrolysis of triglycerides and phospholipids (Baldwin & Allison, 1983). However, unless fats are made inert through chemical or physical processes (e.g. heating or saponification) they might influence to a certain extent rumen environment. In fact, if fed in excess, they may decrease dry matter intake (**DMI**) and they may inhibit microbial growth and rumen fermentation. But fatty acid supplementation may also help to switch VFA production from acetic to propionic acid, and seems to inhibit protein degradation, therefore increasing post-ruminal amino acids availability (Jenkins & Jenny, 1989; Jenkins & Fotouhi, 1990; Emery & Herdt, 1991).

Nitrogen compounds can be metabolised in different ways in the reticulorumen. They can be used as source of energy by the microbiome or they can be use as amino acids source to synthesise microbial protein or, in case of insoluble protein, they overpass the rumen without being modified to be digested in the lower gut. Whether they become energy source or substrates for protein synthesis, the first step of polypeptide metabolism is their hydrolysis to amino acids. Amino acids may then be deaminated with the generation of ammonia and VFA or decarboxylated with the generation of amine and CO_2, the latter reaction being typical of a relative abundance of ready fermentable carbohydrate in the medium (Sanford, 1963; Baldwin & Allison, 1983).

Table 1-1. Major components of rumen microbiome, their substrates and products synthesised (Van Soest, 1994)

Species	Gram classification	Substrates								Products
		Cellulose	Hemicellulose	Pectin	Starch	Sugar	Lipid	Protein	Acids	
Structural CHO fermenters										
Ruminococcus albus	Gram +	H F C	F X							1,2,Et,H_2,CO_2
Ruminococcus flavefaciens	Gram +	H F C	F X							1,2,Su,H_2,CO_2
Fibrobacter succinogenes	Gram -	H F C	H Hm		F Dx					1,2,Su
Butyrivibrio fibrisolvens	Gram -	H F C	F X					F Pr		1,2,4,Et,La,H_2,CO_2
Eubacterium cellosolvens	Gram +	H F C	F X					F Pp		1,2,4,La,CO_2
Pectinolytic species										
Succinivibrio dextrinosolvens	Gram -		F Pn	F Pc						1,2,Su,La
Lachnospira multiparus	Gram +	F Cb		F Pc						1,2,Et,La,H_2,CO_2
Non-structural CHO fermenters										
Bacteroides ruminicola	Gram -	F Cb	F X	F Pc	F S	F Hx		F Pr		1,2,3,Su
Bacteroides amylophylus	Gram -				F S			H Pr		1,2,Su
Selenomonas ruminantium	Gram -	F Cb	F Pn		F S	F Hx	F Gl	F Pr		2,3,4,Su,La,H_2
Streptococcus bovis	Gram +	F Cb		H Pc	F S	F Hx		F Pr		1,2,Et,La
Eubacterium limosum	Gram +	F Cb	F Pn	F Me		F G Fr			F La	2,4
Megasphaera elsdenii	Gram -				F Ml	F Su	F Gl	F Pp	F La	2,3,4,5,6,H_2,CO_2
Lipolytic species										
Anaerovibrio lipolytica	Gram -					F Fr	F Tg	A	F La	2,3,Su,H_2,CO_2
Proteolytic species										
Peptostreptococci sp.	Gram +					Fr		F Pr A		2,4,Br,NH_3,CO_2
Clostridia sp.	Gram +	F Cb	F X		F S	F Sc Fr		F Pr A		1,2,4,Br,Et,La,H_2,NH_3,CO_2
Organic acid fermenters										
Megasphaera elsdenii	Gram -				F S	F Ml	F Gl	F Pp	F La	2,3,4,5,6,H_2,CO_2
Veillonella alcalescens	Gram -								F La	2,3,H_2,CO_2

Legend:

A = amino acids
Br = branched-chain fatty acids
C = cellulose
Cb = cellobiose
CO_2 = carbon dioxide
Dx = dextrins
Et = ethanol
F = ferments and utilises substrate
Fr = fructose
G = glucose
Gl = glycerol
H = hydrolyse substrate but does not utilise products
H_2 = hydrogen
Hm = hemicellulose
Hx = hexose
La = lactate
Me = methanol
Ml = maltose
Pc = pectin
Pn = pentose
Pp = peptides
Pr = protein
S = starch
Sc = sucrose
Su = succinate
Tg = triglycerides
X = xilan
1 = formate
2 = acetate
3 = propionate
4 = butyrate
5 = valerate
6 = caproate

1.1.2 Neural control of rumen motility

The literature cited in the following paragraphs is sparse and dated back to many years ago, however, to the author's best knowledge, the current model of rumen motility derives from these works and there are not recent resources that invalidate or change what was stated.

Ruminal motility is an essential complement to the biochemical processes described above. Forestomach movements provide mixing of the ingesta and its inoculation with rumen microorganism, they also combine rumen content with saliva to ensure VFAs buffering, they prevent the accumulation of VFAs facilitating their absorption through ruminal wall and they allow the elimination of fermentation gases through eructation (Leek, 1969).

1.1.2.1 Reticuloruminal contraction cycles

Within the cyclic events that characterised the forestomach motility of the adult cow, three specialised contraction patterns have been described: primary, or mixing, contractions, secondary, eructation, contractions and rumination (Habel, 1956; Constable, Hoffsis, & Rings, 1990a). The primary cycle, which occurs at the average rate of 60 cycles/hour/day, starts with a biphasic contraction of the reticulum which firstly halves its internal volume and then, it completely collapses its lumen. Subsequently, a simultaneous tensing of cranial, caudal, and dorsal coronary pillars results into a dorsal ruminal sac contraction, which force rumen content into the ventral ruminal sac. Following a short relaxation, the contraction of the caudo-ventral and caudo-dorsal ruminal sacs force rumen content toward the reticulum and cranial sac of the rumen (Habel, 1956; Sellers & Stevens, 1966; Constable, Hoffsis, & Rings, 1990a). The secondary, eructation, cycle occurs independently of the primary contraction and, although its description is less complete than primary cycle, it is generally accepted that it originates in the caudo-ventral blind sac. Following the contraction of the caudo-ventral coronary pillar, also the caudo-dorsal coronary pillar contracts thus moving the gas cap cranially. A rise in the pressure in the cranio-dorsal rumen sac is followed by dilation of the cardia, filling of the oesophagus with gas and its very rapid expulsion through the mouth (Sellers & Stevens, 1966). Rumination is complex process in which ingesta are regurgitated, mixed with saliva and re-chewed and, at last, swallowed again. It starts in the reticulum where specialised epithelial receptors detect the presence of coarse fibre. Regurgitation of the reticular content is associated with an inspiration with close glottis

which creates a negative pressure in the mediastinum. The cardia then relaxes and the oesophagus is rapidly filled with ingesta. Reverse peristalsis moves the bolus in the mouth where it is further chewed (Constable, Hoffsis, & Rings, 1990b).

1.1.2.2 Central innervation of the reticulorumen

Such a complex coordination of voluntary and involuntary movement would not be possible without the action of a system devoted to organise and integrate different afferent inputs in order to generate a harmonised response. The gastric centres, situated in the medulla oblongata, are the structures responsible for this integration (Constable, Hoffsis, & Rings, 1990a). The left and right vagus nerves connect the gastric centres to the effector organs. The two nerves, after leaving the carotid sheath, divide into dorsal and ventral branches; the left and right dorsal branches unite at the level of ninth thoracic vertebra while the left and right ventral branches unite immediately behind the bifurcation of the trachea, thus originating a dorsal and a ventral vagal trunks which pass through the diaphragm (Habel, 1956). Upon entering the abdominal cavity, the ventral trunk gives off several branches which bring neural inputs to part of the reticulum, the ventral parietal surface of the omasum and the abomasum. Following ventral vagotomy rumen motility (mixing contraction, rumination and eructation) is mostly preserved as the major part of the rumen and the omasum are innervated by the dorsal trunk. Dorsal vagotomy affects the normal rumen motility and is generally considered to have greater impact than ventral vagotomy (Habel, 1956).

1.1.2.3 Control of ruminal motility and its modulation

The gastric centres do not appear to generate spontaneous rhythmic stimuli but, on the contrary, to be dependent from external sensory inputs in order to produce periodical vagal discharge. Consequently, frequency, amplitude and duration of the ruminal contraction are determined by the relative extent of excitatory and inhibitory inputs. These may be nervous inputs originating in the gut wall, or the processing results of the higher regions of the central nervous system or even be hormones capable of influencing both central and intrinsic systems (Leek, 1969; Harding & Leek, 1972; Grovum, 1981).

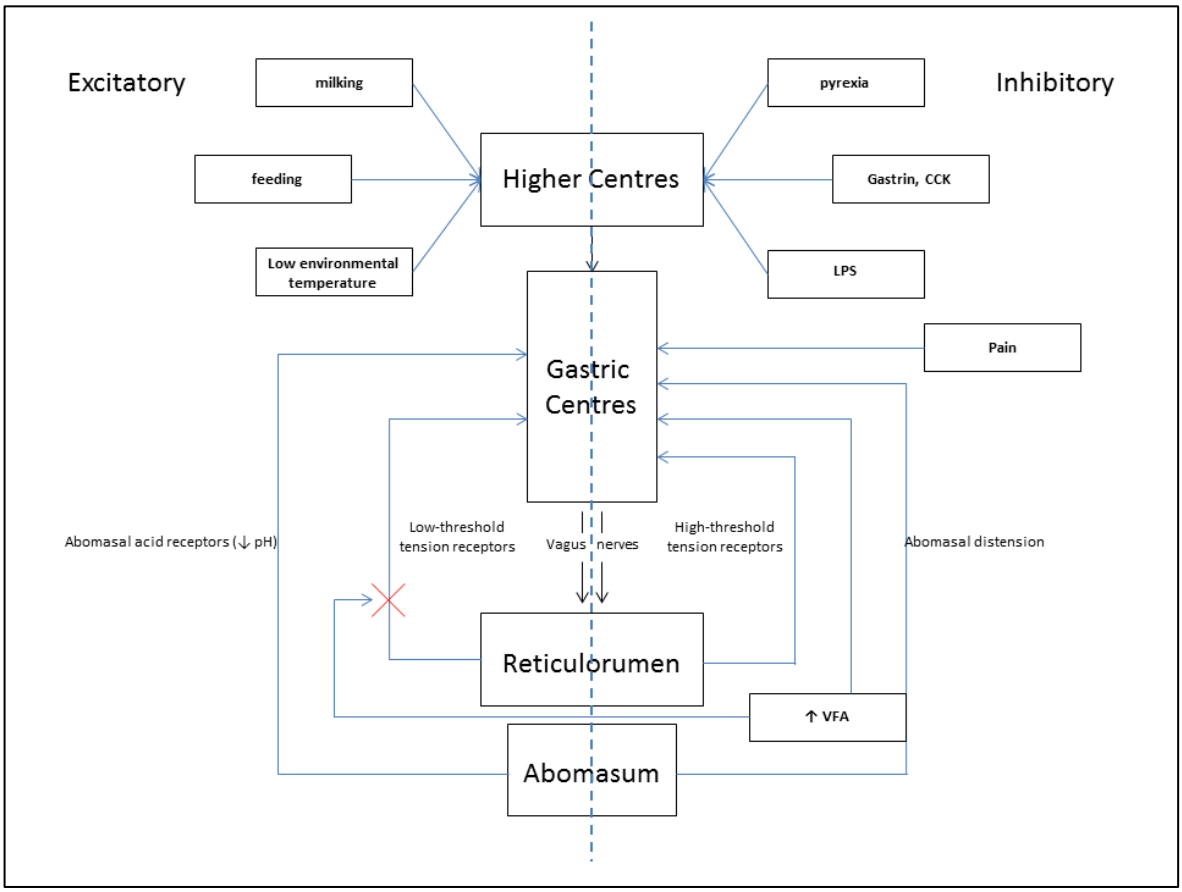
For example, stimuli perceived from sensory organs (e.g. visual, olfactory or acoustic stimuli such as preparation, delivery or smell of the food) and elaborated centrally may increase the frequency of ruminal contraction. Another proof of the existence of a descending influence to the gastric centres was provided by Harding and Leek in 1972. They continued to record period gastric centres activity after the complete resection of both vagus nerves and the spinal cord, transected caudally to the medulla oblongata, in the cervical region, but not when the resection was done cranially, at the level of the pons (Harding & Leek, 1972).

The nature and the effects of the inputs originating from sensory cells located in the gut were thoroughly reviewed by Leek (1969). Tension receptors are most densely located in the medial aspect of the reticular wall and they are connected to the gastric centres through afferent fibres of the vagus nerves. Low threshold (about 4 mmHg) tension receptors are the primary source of the excitatory input to the gastric centres and they provide a continuous stimulus proportional to degree of reticular tension. The effect, mediated by the gastric centres, is an increase of duration and amplitude of the primary cycle contractions. On the opposite site, high threshold (about 20 mmHg) tension receptors respond to a higher degree of reticuloruminal distension decreasing the frequency of ruminal contractions. Under physiological conditions, these receptors do not generate continuous discharges as the low-tension receptors do, but they are activated by the peak of each reticular contraction, leading to its termination. Continuous generation of action potentials by these receptors may be seen in case of pathologic conditions that cause severe ruminal distention, such as rumen impaction or bloat. Acid receptors located in the abomasum also contribute to stimulate reticuloruminal motility. As the pH decreases following abomasal emptying, signals are sent centrally to increase the flow rate of the ingesta (Leek, 1969).

Finally, hormones have been shown to affect reticuloruminal motility. In particular, gastrin and cholecystokinin decreased frequency of ruminal contractions, and, at high dose, gastrin also diminishes contraction amplitude (Grovum, 1981). Although it is possible, during parasitic infestation, to have high blood levels of gastrin and cholecystokinin, which helps to explain the decrease in appetite and ruminal movement observed, the physiological role of these hormones in modulating reticuloruminal motility is still uncertain (Constable, Hoffsis, & Rings, 1990a; Onaga, 2007). Also VFA have been shown to affect rumen motility, in a hormone-similar manner, in both vagus intact and vagotomised sheep (Gregory, 1987). In a series of experiments, increasing concentration of VFA in the rumen, decreased ruminal contraction and two mechanisms, not mutually exclusive, have been

proposed. The early decrease in amplitude of the primary, followed by the secondary, ruminal contractions is believed to be primarily the result of vago-vagal reflex, through the activation of ruminal acid-sensitive receptors. With the increase of the molar concentration of VFA in the reticulorumen, VFA may also act independently from the SNC, inhibiting the discharge of the low-threshold tension receptors and contributing in this way to the ruminal stasis observed (Gregory, 1987).

Figure 1-1. Central modulation of reticuloruminal motility



The figure represents the interactions among those physiological or pathological factors that contribute to modulate reticuloruminal motility at central level. Figure adapted from Constable and colleagues (1990a).

1.2 Ruminal acidotic disorder

1.2.1 Carbohydrates dysmetabolism and its consequences

It might be reductive and erroneous to conceive the rumen solely as an organ of the digestive system. Instead the rumen could be imagined as a dynamic ecosystem that coexists within the host, cooperating with it with mutual advantages. The peri-parturient or transition period, hereafter referring to the last three weeks before calving and the first three weeks after calving, is the period in which the dynamism and the resilience of the rumen and the cow is most deeply challenged. The primary challenge is a sudden and marked increase of the nutrient requirements of the animal, at a time when DMI lags far behind. During this period, coefficients of variation (**CV**) of DMI between animals are in the range of 30% to 40%, while after lactation and the time of peak DMI, the CV are between 6% and 10% (Drackley, 1999). In order to compensate for the decrease in DMI, the most efficient use of energy and nutrient must be pursued and this has led, to accepted management practices that include high amounts of highly fermentable carbohydrate in diets of freshly calved cows. NSC are quickly and completely fermented in the ruminal environment with the immediate consequence of a sudden increase in VFA molar concentrations in the reticulorumen. This sudden increase in VFA presents another strong challenge to the rumen that, during the dry period, has usually become adapted to a highly fibrous low-energy diet. In the ruminal environment VFA are mostly dissociated as their pK_a (average: 4.8) is lower than ruminal pH, therefore, if the VFA ingestion and production overcome the capability of rumen epithelium to absorb them, or the buffering potential of phosphate and bicarbonate systems, a large amount of protons is released into the ruminal medium (Aschenbach et al., 2011). As a result, ruminal osmolality increases and ruminal pH becomes more acidic. These changes, with the relative abundance of CHO as a fermentative substrate, favour the non-structural CHO fermenters population with an overall reduction in bacterial diversity (Khafipour et al., 2009; Plaizier et al., 2017; Mu et al., 2019). If these conditions persist, *Streptococcus bovis* outgrows other species switching the starch metabolism toward the production of ethanol and lactate. Lactate is a ten times stronger acid than VFA and its production leads to a further increase in ruminal osmolality and decrease in ruminal pH (Russell and Hino, 1985). If the pH decreases below 4.5, *S. bovis* growth is eventually inhibited and very acid tolerant bacteria belonging to the genus *Lactobacillus* take over, resulting in the overt signs of clinical acidosis (Underwood, 1992). However, early stages of the biochemical process described above may have no detectable clinical consequences and may be subtle, very

difficult to recognise in a typically busy farm environment. Historically, these symptoms have been grouped under a syndrome named subacute ruminal acidosis or SARA.

Depression of feed-intake, or erratic appetite, are quite consistently reported as clinical signs of SARA (Plaizier et al., 2008; Humer et al., 2018a). Several possible underlying pathophysiological mechanisms have been hypothesised but no definitive mechanism has been described yet. A first possible explanation involves the feed-back system that exists between the rumen and the SNC. In response to the osmotic disequilibrium generated by the high molar concentration of VFA, water diffuses from intra- and extra-cellular spaces into the rumen. As the pressure applied from the rumen content to the reticuloruminal wall increases over 20 mmHg, it activates high-threshold tension receptors which send inhibitory inputs to the gastric centres situated in the medulla oblongata. In turn the gastric centres inhibit reticuloruminal contractions slackening rumination, fermentation and emptying process (Leek, 1969). As a result the rumen continuously signals to satiety centres in the hypothalamus limiting animals' appetite (Allen, 2000; Sjaastad, Hove, & Sand, 2003). The acid sensitive epithelial receptors stimulated by a high concentration of VFA may also send directly inhibitory inputs to the gastric centres, causing a reduction in appetite (Constable, Hoffsis, & Rings, 1990a). The second major group of proposed mechanisms for some of the effects of SARA revolve around systemic inflammation, which has been detected as an elevation of acute phase protein, likely as a consequence of high levels of lipopolysaccharide (**LPS**) accumulation in reticuloruminal fluid. Bacterial LPS are lipophilic component of the outer membrane of gram-negative bacteria which are released following cell disintegration and lysis. Lysis of gram-negative bacteria in case of carbohydrate engorgement may be subsequent the sudden decrease in ruminal pH or the increase ruminal osmolality. It has been hypothesised, as described in detail in the following section, that the inflammation consequent to LPS absorption may be, at least in part, responsible for the feed intake depression (Plaizier et al., 2008).

Another clinical sign often associated with SARA is diarrhoea (Plaizier et al., 2008; Oetzel, 2017). Diarrhoea is usually related to the reduced capability of rumen to digest fibre during episodes of SARA due to the depletion of structural CHO fermenters that begin when rumen pH drops below 6.0 (Plaizier et al., 2008; Aschenbach et al., 2011). The CHO that escapes rumen degradation may be fermented in the hindgut with increase in the acid load, osmolality and, hence, diarrhoea (Plaizier et al., 2008).

Low rumen pH may also affect the integrity of the structure of the ruminal epithelium and cause rumenitis (Oetzel, 2017). The rumen has a stratified squamous epithelium composed of four different layers which are, from the lumen to the basal lamina, the stratum corneum, the stratum granulosum, the stratum spinosum and the stratum basale (Graham & Simmons, 2005). The stratum corneum, the layer that faces the content of rumen, consists of multiple layers of keratinised cells. The stratum granulosum is formed by cells tightly joined together by junctional complexes called desmosomes and it is the layer that confers structure and solidity to the rumen wall. The stratum spinosum is the most metabolically active among the four and most of the butyrate absorbed is converted in β-hydroxybutyrate (**BHB**) by this population of cells. The stratum basale, finally, regenerates the above-mentioned layers, being the stratum with highest mitotic rate. The newly formed cells, move from the basal lamina toward the lumen and, gradually, they undergo a keratinisation process (Sjaastad, Hove, & Sand, 2003; Steele et al., 2011). Parakeratosis, the excessive thickening of the stratum corneum, is the most common abnormality linked with rumen acidosis. It has been hypothesised that a parakeratotic epithelium would decrease the VFA absorption rate, therefore causing a further increase of osmolality and reduction in pH. The excess of acidity and osmolality finally cause the breakage of the epithelial integrity, with consequent translocation of rumen bacteria to the portal blood (Steele et al., 2009; Oetzel, 2017). Milder forms of acidosis, however, may not cause a thickening of the stratum corneum but an overall reduction in thickness and organisation of the three underlying layers. In particular Steele and colleagues (2011) found that a number of genes coding for desmosomes were downregulated during a mild acidotic event. It has been hypothesise that the combined effect of a thinner and less structured epithelium may allow the transfer of bacteria and LPS from the rumen environment to the systemic circulation (Steele et al., 2011). With more severe damage to the gut, the *stratum corneum* can detach from the underlying layers and the ruminal wall will become subjected to bacteria colonisation (Nagaraja & Lechtenberg, 2007; Steele et al., 2009). Once gut integrity becomes so compromised, bacteria can gain access to the portal circulation and infect the liver causing liver abscessation. Aside from the economic loss of liver rejection at the abattoir, animals with widespread lesions in the liver parenchyma, may have lower performance levels due to reduced feed intake and reduced feed efficiency (Nagaraja & Lechtenberg, 2007). It is important to mention that this translocation may happen also, or even more often, in the hindgut. The large intestinal epithelium is simpler and thinner compared with the rumen and such histological differences may result in a barrier more prone to be damaged by unusual pH or osmolality (Li et al., 2012).

A systemic inflammatory process mediated by xenobiotics or bacteria may follow the disruption of the GI barrier. Lipopolysaccharides and biogenic amines are xenobiotics derived from the symbiotic microbiome, and they have been extensively studied in recent years for their possible involvement in the inflammatory process. As mentioned above, LPS are component of the outer membrane of gram-negative bacteria which are released following cell disintegration and lysis. An increase in the concentration of ruminal LPS following high concentrate diets have been reported (Gozho et al., 2005; Zebeli & Ametaj, 2009; Li et al., 2012) and, recently, the possibility of their translocation from the digestive tract to internal body compartments has been hypothesised and demonstrated (Khafipour, Krause, & Plaizier, 2009a; Guo et al., 2017). Following the interaction with mononuclear cells and LPS-binding proteins (**LBP**), LPS activates the nuclear factor-κB (**NF-κB**) pathway through the mediation of a class of transmembrane proteins known as toll-like receptors (**TLR**). The resulting activation of the inflammasome lead to the synthesis of inflammatory mediators such as tumour necrosis factor-α (**TNF-α**), interleukin-6 (**IL-6**) and interleukin-1β (**IL-1β**), by mononuclear cells which, in turn, induce the hepatic synthesis of serum amyloid-A (**SAA**) and haptoglobin (**Hp**), neutrophilia and lymphopenia (Ulich et al., 1989; do Nascimento, Hunter, & Trayhurn, 2004; Zhang et al., 2005; Plaizier et al., 2012). Biogenic amines, such as tyramine, histamine, putrescine, tryptamine and methylamine, have also the potential to trigger an inflammatory reaction (Sun et al., 2017). Wang and colleagues (2013) found that concentrations of histamine and other biogenic amines increased in rumen fluid and peripheral blood following a dietary protocol that was successful to induce SARA. The normal metabolic fate of aminoacids is to be deaminated however decarboxylation can occur in condition of relative abundance of carbohydrate out by carbohydrate fermenting bacteria such as *Streptococcus bovis* and *Lactobacillus* spp. (Sanford, 1963; Wang et al., 2013). Histamine, as well as LPS, exerts its action activating the nuclear factor-κB pathway (Sun et al., 2017); whereas other amines, or other inflammatory pathways, are involved and in what extent, need still to be determined.

It has been also hypothesised, although to the best author's knowledge experimental evidences are lacking, that these xenobiotics may be implicated in the pathogenesis of laminitis in cattle (Nocek, 1997).

1.2.2 Clinical definition of SARA and its criticisms

The variety of nonspecific clinical signs, often covert in the initial phases of this complex internal disease, pushed researchers and academicians to identify a suitable clinical test capable, reliably and repeatedly, to identify the disease. In the mid nineties, researchers from the University of Wisconsin recommended the measurement of pH from ruminal fluid collected by rumenocentesis as valuable tool to diagnose SARA. In 1994, Nordlund and Garrett described rumenocentesis applied to SARA diagnosis, and the same group in 1995 introduced the concept of groups at risk (Nordlund & Garrett, 1994; Nordlund, Garrett, & Oetzel, 1995). Since the very beginning it was clear that the definition of an appropriate cut-off between "normal" and "abnormal" pH would have been problematic, and this test, like most laboratory tests, should have been interpreted alongside history and clinical exam findings (Nordlund & Garrett, 1994). Eventually, in 1999, the cut-off point of 5.5 was established together with sample group size and a critical number of positive results. If at least three out of twelve animals tested had rumen pH < 5.5 then the probability to correctly classify the group as at risk of SARA, varied from about 50% to 0.98% according with the prevalence of the disease (Garrett et al., 1999). It is important to note that the intended outcome for this test was not to diagnose the disease but classify a group of animals as "at risk" to develop it. As the imbalance between the rate of acid production and the rate of acid absorption or proton buffering is considered the primary event resulting in those diverse and multiple consequences described above, ruminal pH appeared to be a very appropriate clinical parameter to discriminate between healthy and sick groups. Notwithstanding that the accuracy of the test dramatically decreased at an intermediate level of disease prevalence, and despite Wisconsin researcher recommendations, in the following years the clinical definition of the disease heavily relied on ruminal pH (Nocek, 1997; Krause & Oetzel, 2006). However, over the years, criticisms arose also about the low reproducibility of the diagnostic process. For example Duffield and colleagues (2004) showed the variability of rumen pH across sampling sites within the rumen in 16 fistulated cows. They also suggested higher critical thresholds (pH ≤ 5.9 and pH ≤ 6.2) for those samples taken with oro-ruminal probe instead of rumenocentesis technique. Other factors, such as circadian and seasonal changes, can affect a single point measurement. Indeed regardless the feeding management, pH has repeatedly shown to drop during the day and to start raising up in late afternoon or evening (Kimura et al., 2012; Antanaitis et al., 2016; Denwood et al., 2018). Moreover, season of the year appears to affect rumen pH, which is lowest in summer and highest in fall and spring (Antanaitis et al., 2016). Finally, parity has been shown to affect animal's response to the

diet. For instance, Humer and co-workers (2015) found that, within the same diet, multiparous cows have been shown to have shorter bouts of low ruminal pH compared with first lactating cows, maybe due to their previously adapted rumen microbiome or the longer time they usually spend ruminating (Humer et al., 2018b).

Therefore, several researchers tried to improve the accuracy of the definition of the disease by combining other clinical parameters with rumen pH and proposing different pH thresholds. For instance Gozho and colleagues (2005) defined SARA as a condition in which ruminal pH drops below the threshold of pH 5.6 for more than 3 hours/day, as this was associated with signs of systemic inflammation. Other authors have suggested a pH value of 5.8 as the cut off because, although pH of 5.8 might not influence the cow's general inflammatory status, ruminal efficiency is likely to be decreased due to the shift of ruminal microbiome toward lactate-utilizing bacteria (Aschenbach et al., 2011). Zebeli and colleagues (2008) adopted the last criterion stating that the probability of cows experiencing SARA increases if ruminal pH remains below 5.8 for longer than 6 hours/day. More recently there has been increasing interest in the metabolites produced by the microbiome during those periods considered to be at risk for SARA. More precisely, Saleem and colleagues, using molecular techniques such as proton nuclear magnetic resonance spectroscopy, Gas Chromatography – Mass Spectrometry, and direct flow injection tandem mass spectrometry, described and quantify the shift in metabolites in cows fed at increasing quantity of barley grain (0%, 15%, 30% and 45% of the diet DM basis). These researchers showed that increasing inclusions of barley grain in the diet, led to a higher concentration of potentially harmful metabolites such as methylamine, putrescine, cadaverine, LPS, ethanol, xanthine, and others (Saleem et al., 2012).

1.2.3 Prevalence of SARA across European dairy herds

Prevalence of a disease can be defined as the number of cases of the disease that occur in a particular population at a given time. Not many fields study have attempted to determine SARA prevalence, but, despite a substantial improvement in understanding the pathogenesis of the disease, until the most recent years, every attempt was still based on single point measurement of ruminal pH. Studies have been conducted in different European countries and data are available from Austria, Germany, Greece, Ireland, Italy, Netherland, Poland and UK. With the unique exception of the Austrian researchers, who collected ruminal fluid by a stomach tube, all the other studies used rumenocentesis as a mean to obtain ruminal samples. Two main measures have generally been obtained in these studies, prevalence of SARA at cow level and prevalence of SARA at herd level. In those studies where rumenocentesis was used, prevalence of SARA at cow level was defined as the number of cows with rumen pH $\leq$ 5.5 divided by the total number of cows sampled and ranged from 11% to 20%. At herd level, SARA was generally diagnosed if at least 25% of the animals sampled in a single group had ruminal pH $\leq$ 5.5 and with this criterion prevalence across herds ranged from 17% to 44%. Because of the different sampling technique, in the study conducted in Austria, authors set pH threshold at 6.0. Out of the 258 samples obtained none was < 6.0 therefore both cows and herd prevalence are 0% (Steiner & Wittek, 2017). The authors concluded that the peculiar housing and feeding management implemented in Austria might explain the lower risk of SARA detected in herds capable to achieve relatively high milk production. Tables 1.2 and 1.3 show the detailed results at cow and herd level respectively.

Table 1-2. Prevalence of SARA across European dairy herds - cow level

Country	Sample size	At risk of SARA pH ≤ 5.5		Marginal results 5.6 ≤ pH ≤ 5.8		Negative pH > 5.8		Reference
		No of cows	Prevalence	No of cows	Prevalence	No of cows	Prevalence	
Austria	258	0	0%	1*	0.4%	257	99.6%	(Steiner & Wittek, 2017)
Germany	320	63	20%	104	33%	148	48%	(Kleen, Upgang, & Rehage, 2013)
Greece	153	24	16%	25	16%	104	68%	(Kitkas et al., 2013)
Ireland	144	16	11%	60	42%	68	47%	(O'Grady, Doherty, & Mulligan, 2008)
Italy	-	-	-	-	-	-	-	(Morgante et al., 2007)
Netherland	117	27	14%	33	17%	57	49%	(Kleen et al., 2009)
Poland	213	30	14%	29	13%	154	73%	(Stefańska et al., 2017)
UK	244	64	26%	-	-	180	74%	(Atkinson, 2014)

*In the Austrian study pH threshold was set at 6.0 and only one cow had pH = 6.0

Table 1-3. Prevalence of SARA across European dairy herds - herd level

Country	Sample size	At risk of SARA ≥ 25% of cows with pH ≤ 5.5		Marginal results ≥ 33% of cows with pH ≤ 5.8		Negative ≤ 25% of cows with pH ≤ 5.5 and ≤ 33% of cows with pH ≤ 5.8		Reference
		No of herds	Prevalence	No of herds	Prevalence	No of herds	Prevalence	
Austria	20	0	0%	0	0%	20	100%	(Steiner & Wittek, 2017)
Germany	26	11	42%	12	46%	3	12%	(Kleen, Upgang, & Rehage, 2013)
Greece	12	4	32%	1	8%	7	60%	(Kitkas et al., 2013)
Ireland	12	3	25%	6	50%	3	25%	(O'Grady, Doherty, & Mulligan, 2008)
Italy	10	3	33%	5	50%	2	20%	(Morgante et al., 2007)
Netherland	18	3	17%	5	27%	10	56%	(Kleen et al., 2009)
Poland	9	4	44%	0	-	5	56%	(Stefańska et al., 2017)
UK	29	13	45%	6	21%	10	34%	(Atkinson, 2014)

1.2.4 Differential effects of dietary composition on rumen health

Chemical composition of the diet has the potential to positively or negatively affect rumen health and nutrient utilization through the interaction with rumen microbiome. Starches and sugars belong to the same chemical class of nutrient, but sugars are generally characterised by a faster fermentation rate within the rumen as a result of their inferior chemical complexity. The Cornell Net Carbohydrate and Protein System assumes that sugar fermentation rate ranges from 200% h^{-1} to 400% h^{-1} compared with a degradation rate of starches that varies from 30% h^{-1} to 400% h^{-1} (Lanzas et al., 2007). Sugars therefore, to a higher degree compared with starches, may have the potential to cause a sudden increase in ruminal VFA and trigger SARA.

In many studies, inclusion of sugars in the diet increased or tended to increase DMI and, because they are a source of energy that is completely available to rumen bacteria, they improve ruminal utilisation of non-protein nitrogen (Broderick & Radloff, 2004; DeFrain et al., 2004; Martel et al., 2011; Chibisa et al., 2015; Gao & Oba, 2016). Furthermore, more often than starches, sugars are converted to butyrate rather than propionate and their inclusion in cows' diet have often led to an increase of molar proportion of butyrate and sometimes to a decrease in propionate (Martel et al., 2011; Mojtahedi & Danesh Mesgaran, 2011). Effects of sugars on acetate production is less consistent with acetate increasing after sugars inclusions in some trials and decreasing in some others (Chamberlain et al., 1985; DeFrain et al., 2004; Martel et al., 2011; Mojtahedi & Danesh Mesgaran, 2011). Due to variability in sampling times and techniques used, results relative to rumen pH have been very inconsistent and often contradictory. In several studies, dietary sugar addition did not cause significant changes in pH values (Broderick and Radloff, 2004; DeFrain et al., 2004; Broderick et al., 2008; Chibisa et al., 2015) while it decreased mean pH values in some others (Chamberlain et al., 1985; Gao and Oba, 2016) or even increased (Martel et al., 2011) or tended to increase (Penner and Oba, 2009; Penner et al., 2009) average pH values in some recent trials. Furthermore, none of the studies cited investigated the systemic effect that these two sources of carbohydrate had in terms of inflammatory response.

1.3 Continuous monitoring of cattle health

Regardless of cause, health disorders may substantially affect cow welfare and heavily jeopardise the efficiency and the productivity of a dairy unit (Stangaferro et al., 2016a, 2016b). Monitoring programs that aim to identify sick animals are often adopted by commercial farms firstly because early disease detection is a key aspect of a successful therapy, and secondly because accurate information about prevalence and incidence of a certain disease is often required to implement preventative or control actions. Monitoring programs may include monitoring of feeding behaviour or performing cow-side tests that may involve collection of body fluid, percussion or auscultation of the animal and keeping detailed records of all the above (LeBlanc, 2010). Performing these tasks for a large number of animals can be time-consuming and may disrupt workers' and cows' routines, hence the interest in developing sensors capable to generate data for accurately describing animals' health status and its modification. Many different devices are currently available, each developed to monitor one or more aspect of an animal's physiology or behaviour, with the aim to early detect problems related to lameness, mastitis, metabolic and infectious disease (Rutten et al., 2013; Stangaferro et al., 2016a; Alsaaod, Fadul, & Steiner, 2019). Among the sensors, those developed to monitor and diagnose metabolic diseases are less developed (Rutten et al., 2013). A great part of the challenge is due to the intrinsic nature of these diseases, often characterised by multifactorial aetiology and involvement of multiple organs and systems.

Devices that monitor DMI have been a focus of research. DMI over the transition period have been related to both depth and duration of negative energy balance (**NEB**) in the post-partum period and NEB has been linked with a higher disease incidence shortly after calving (Drackley & Cardoso, 2014). Therefore, an automated system that monitors DMI has been developed and validated in the Netherlands. The device uses a 3-axis accelerometer to detect jaw movements and hence recognising eating time. A more pronounced circular motion of the jaw allowed differentiation between eating and rumination (Van Erp-Van der Kooij, Van De Brug, & Roelofs, 2016; Hut et al., 2019).

In the same way longer time spent ruminating has been associated with lack of stress, pain, anxiety and improved cows' health (Herskin, Munksgaard, & Ladewig, 2004; Bristow & Holmes, 2007; Stangaferro et al., 2016a). Rumination can account up to 6-7 hours in a day, and by rumination bovines crumble coarse plant structures, mix the cud with the saliva and

its enzymes, and expose ground-up material and cell solutes to microbial digestion (Phillips, 1993). Recently a device capable of identifying time spent ruminating was validated both under research and commercial environmental condition (Schirmann et al., 2009; Ambriz-Vilchis et al., 2015).

Continuous monitoring of reticuloruminal temperature has been tested for detection of respiratory disease in bulls under intensive fattening management condition. The device consisted on a bolus including a temperature sensor, internal lithium-ion battery and a microprocessor for the control of circuit functions and timing (Timsit et al., 2011). Similar sensors have been tested for detection of post-partum diseases, pneumonia and mastitis in dairy cows as well as for detection of SARA (AlZahal et al., 2008; Adams, Olea-Popelka, & Roman-Muniz, 2013).

Redox potential (**Eh**) of reticuloruminal environment has the potential to be an extremely useful clinical parameters for continuous monitoring. Oxidation-reduction reactions are essential for microorganism life and each microorganism is adapted to specific Eh range. Eh has therefore the potential to modify dominant class of bacteria and influence chemical pathways for food degradation, hence increasing or decreasing rumen energetic metabolism efficiency (Huang et al., 2018). To date, few studies have investigated *in vivo* Eh and none has established clear cut-off values or investigated the diagnostic value of this parameter (Richter, Křížová, & Třináctý, 2010; Qin et al., 2017).

1.3.1 Indwelling sensors applied to SARA diagnosis

To apply continuous monitoring for the diagnosis of SARA, the main focus has been on ruminal pH. Enemark and co-workers (2003) described continuous pH monitoring on two cannulated dairy cows and showed substantial agreement between the indwelling probe and traditional pH-metre. The authors concluded that similar sensors would be reliable and useful, and suggested that focusing on pH dynamics (i.e. length of period in which pH is below a certain threshold) rather than simple pH nadir may be a better diagnostic marker for SARA. Other authors independently developed the same concept and new metrics such as daily average pH, time < pH, area (time × pH) under pH were proposed (Krajcarski-Hunt et al., 2002). Gozho and colleagues (2005) evaluated these new methods of measurement simultaneously investigating the acute phase response in association with high concentrate diets. They concluded that SAA was a more sensible marker compared to Hp and they suggested that 180 minutes/day (**min/d**) spent with rumen pH below 5.6 as threshold for SARA. The rationale beyond these metrics lies over the fact that ruminal pH is not a constant value but varies according to time of the day. Therefore, these more "dynamic" parameters have been perceived to better describe acid-base ruminal equilibrium. However, the ability of these new metrics too, to provide clear boundaries between physiology and pathology has recently been challenged. In a series of studies conducted at University of Manitoba, cows fed with two different dietary protocols, the first high in NSC, forage:concentrate ratio (**F:C**) 40:60, the second low in physically effective NDF (**peNDF**), resulted in both being positive for SARA according to the above-mentioned pH metrics (Khafipour, Krause, & Plaizier, 2009a, 2009b). Moreover, the diet low in peNDF resulted in a longer time spent below the threshold. However, in the two groups of animals, acute phase proteins (**APP**) showed completely opposite dynamics. Animals fed with the high grain diet had a marked increase, between 2- and 8-fold, of all the APPs, while animals fed low peNDF diet had a consistent reduction in all of the APP measured. Very recently two new methods of analysis of pH datasets were proposed by Denwood, Jonsson and co-workers (2018; 2019). In the first analysis, the focus of the observation was the absolute extent of variation of observed pH values from a predicted pH curve, and variation in the absolute residuals was associated with reduced milk production and reduced DMI. This method of analysing pH datasets changed the emphasis from arbitrarily defined pH values or thresholds that must be applied to all the animals in all conditions, to the output value of a statistical model based on an individual animal's previous pH data history (Denwood et al., 2018). The last work applied indwelling sensors to the herd context evaluating at the same time the performance of the sampling

strategies suggested by Garrett et al. (1999). The study exhibited the role diurnal cycle along with feeding routine in modifying rumen pH, and recommends to change sampling time from 5-8 hours after delivery of total mixed ration (**TMR**) to the interval between 18:00 and 02:00 regardless of the time when feed is delivered. They also suggested that a minimum of nine boluses are necessary to obtain a reasonable approximation of herds mean pH (Jonsson et al., 2019).

Despite the great expectation and large improvement in SARA understanding allowed by the spread of pH boluses in research and commercial environments, these sensors are still showing important limitations. The interpretation of the results requires a large amount of work and efforts and the debate is still ongoing on the subject. Moreover, the cost and the limited lifespan due to sensor drift preclude, at the moment, their routinely use on farm as monitoring tool.

1.3.2 Use of indwelling motion bolus to measure rumen motility

The interactions among physiological and pathological factors that contribute to modulate reticuloruminal motility have been described in section 1.1.2 and are summarised in Figure 1–1. It is easily understood from that figure how many conditions can affect rumen motility and, diseases, of course, will exert their effect by generating fever and pain. SARA influences ruminal motility and, although the mechanism has not been fully elucidated, several different biological pathways have been proposed. For instance, high ruminal VFA concentrations can draw water into the lumen, causing an increase of the internal pressure and a continuous stimulus of high-threshold sensor receptors. High VFA concentration can also affect rumen motility, in a hormone-similar manner acting directly at level of the gastric centres. In the end, the inflammatory process, and the relative mediators, caused by SARA may be in part responsible for the decrease of rumen motility associated with SARA. Very recently two studies have independently developed and tested two boluses capable of measuring reticulo-ruminal motility (Nogami et al., 2017; Hamilton et al., 2019). The sensors were able to identify periods of rumen atony generated by an intramuscular injection of xylazine (Nogami et al., 2017) and to correctly identify rumination periods and quantify the overall animal activity (Hamilton et al., 2019). These sensors are much cheaper compared with pH boluses; moreover they do not need to be recalibrated and their battery consumption is substantially lower, overcoming in this way some of the issues mentioned above.

1.4 Scope of the thesis

As with many other industrial sectors, the dairy industry has faced important challenges in the past years. The requirement for a more efficient, environmentally sustainable and animal-friendly method of production is becoming pressing, and farmers alongside practitioners and academic vets must work to achieve these goals. The improvements achieved in the understanding of ruminants' physiology and nutrition already resulted in a significant enhancement of health and production performance and recent scientific developments are opening new promising areas of investigations.

Dairy cow nutrition may, with good reason, considered an evolving field. Over the entire 20th century, the main criterion for evaluation of nutritional management was increased milk production: a better diet was recognised as such, if it performed better than another in terms of milk output. If no differences were detected, the diet with the lowest feed and labour inputs tended to be preferred as it was considered to be more efficient (Bradford, Yuan, & Ylioja, 2016). However, recent advances have the potential to change our approach to cattle nutrition and, to a certain extent, to affect farm nutritional management. For example, although much evidence indicates that the reticulo-ruminal microbiome actively adapts to exogenous stimuli such as different diet types or diet components, it has been shown that inter-animal differences exist under the same dietary regime, suggesting that host-bacteria interactions play an important role in determining rumen ecology (Firkins & Yu, 2015). Modifications of the microbiome directly affect the metabolites of ruminal fermentation and, ultimately, the feed efficiency. A more sophisticated view should also consider that nutrients, or their metabolites, can act as ligands for cellular receptors and diet type may impact epigenetically on gene expression. For instance volatile fatty acids, beyond being the main energy source in ruminants, mediate a wide range of physiological processes such as rumen papillae development and insulin and glucagon secretion, while a high concentrate diet has been shown to modify the degree of acetylation of histone H3 (Wang et al., 2009; Dong et al., 2014). Furthermore, important links exist between nutrition and the immune system. The first and more obvious is the energetic cost of the immune response: synthesis of acute phase protein, leukopoiesis and increase in body temperature are energy demanding processes that, in the event of sickness, are prioritised over production and reproduction performances (Lochmiller & Deerenberg, 2000). Different interactions are provided by molecules, such as β-hydroxybutyrate or vitamin D, that, acting as ligands, exert a direct effect on the regulation of the immune system (Nelson et al., 2012; Youm et al., 2015). For example, Vit. D acts on

the innate immune system of cattle, enhancing monocytes response via toll-like receptor 4 (TLR4). Also, monocytes exposed to LPS and Vit. D increased inducible NO synthase and RANTES/CCL5 genes expressions compared to monocytes exposed solely to LPS. On the opposite side, BHB has been shown to suppress the activation of the inflammasome exerting an inhibitory function on inflammation. Accounting for all these aspects will change the classical view of cattle nutrition. In fact, the physical constraint represented by rumen capacity, and the necessity to achieve a balance between DMI and persistence of food in the gastrointestinal tract, will likely make it difficult in the future to achieve further gains in milk output. Therefore, focusing on bacteria-host interactions, nutraceutical properties of feeds, on the links between nutrition and immune or endocrine systems may provide, in the long run, better chances to breed healthier and more resilient animals (Bradford, Yuan, & Ylioja, 2016).

Being less than half a century long, the development of sensor systems may be considered among the newest frontiers of cattle medicine. Automated milking systems were probably the first systems introduced in dairy farms in the early 1990s, followed by systems developed to detect and measure differences in cows' activity level, behaviour, milk conductivity, milk concentration of certain hormones or metabolites, ruminal pH and body temperature (Rutten et al., 2013). It should be noted that conception and development of a given sensor is only the first stage of making a tool with the potential to interact with, and possibly to improve, farm management systems. An algorithm for interpretation of sensor's data must follow in order to provide information about the individual animal. A third level of complexity is to produce advice based on sensor data analysis and possibly other information not necessarily related to the device itself. Finally, following the initial measurement and its interpretation, a decision has to be taken regarding cow or herd health (Rutten et al., 2013). Figure 1–2 summarise and provide some examples of the framework proposed by Rutten and co-workers (2013) to describe sensor systems. In 2013, the sensors systems reviewed by Rutten and his co-authors, were for the majority related to fertility (48 systems; 35% of the total) followed by locomotion and mastitis (38 and 37 systems respectively; 27% of the total) and metabolic disease (16 systems; 12% of the total) as shown in Figure 1–3. Fertility and mastitis related systems were the ones more developed with 34 and 36 devices operating at level two while only 18 and five systems respectively operated at this level for locomotion and metabolic diseases. This gap between the number of devices that investigate fertility, mastitis and lameness, and the number of devices related to metabolic diseases might be due

to the fact that metabolic problems have studied for a relatively shorter time compared to others and their nature is complex and varies along with the disease considered (Rutten et al., 2013).

Figure 1-2. Sensors' evaluation framework as described by Rutten et al. (2013)

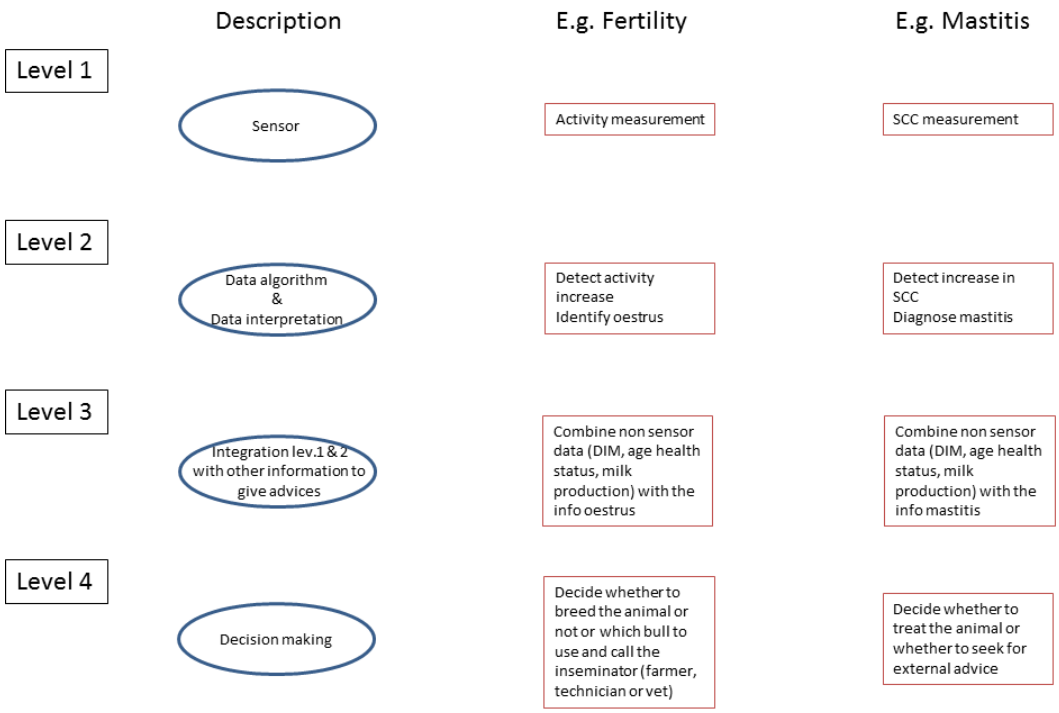
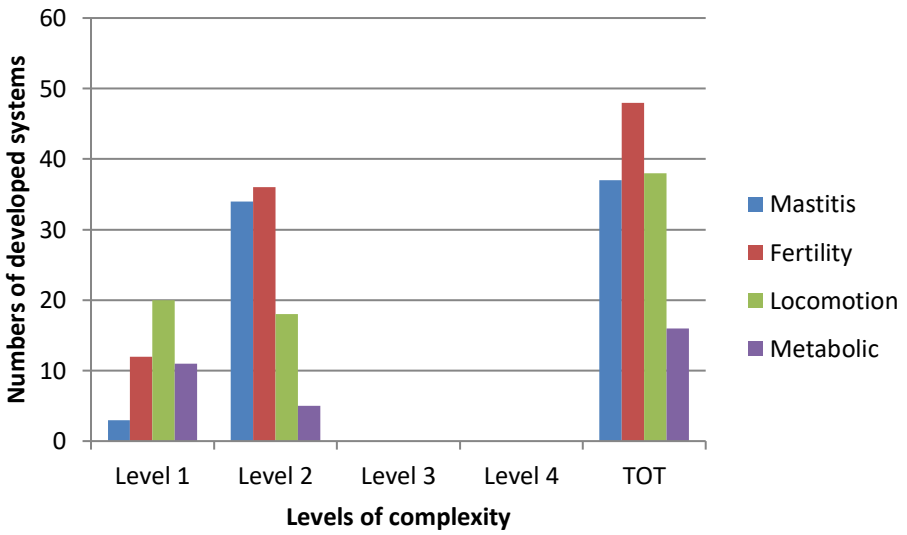


Figure 1-3. Stage of development of dairy sensor systems



Total number of sensor systems developed and the relative number of systems able to work at different levels of complexity. Figure adapted from Rutten et al. (2013).

Subacute ruminal acidosis is a common metabolic pathology in high yielding dairy cows traditionally linked with one or more daily bouts of low ruminal pH. The high degree of alteration that SARA causes to rumen microbiome and metabolism effects animal welfare and productivity, sometimes without causing overt or specific clinical signs. Therefore an interest in tools capable of monitoring clinical or para-clinical parameters has developed in recent years (Plaizier et al., 2008; Oetzel, 2017; Humer et al., 2018a). The following manuscript is a description of the research trial conducted at the University of Glasgow to investigate some specific points of this complicated and fascinating subject:

1. Although different SARA induction models have been developed over the years, the specific dietary components have not been emphasised. However, based on the discussion above, it seems logical to hypothesise that different feedstuffs with similar expected levels of highly fermentable carbohydrates will have differential effects. Hence, the first aim of our study is to compare the systemic effects of two iso-energetic and iso-nitrogenous diets, designed to induce SARA, in which the rapidly fermentable carbohydrate are starch and sugar respectively. Particularly we hypothesise that, being fermented at faster rate, sugar would have led to a more severe effect on rumen pH and overall health status.
2. The lack of uniformity in the definition of the pathology, and its variability in the clinical presentation make the diagnosis of SARA very complex. Therefore, the second aim of our study is to describe the challenge diets and their systemic effects, applying different models in order to evaluate and compare their relative efficacy to describe rumen acidotic disorders and to detect animals affected by these disorders. Specifically we expect that the model developed by Denwood and colleagues (2018) would correlate better ruminal pH results with other biomarker of cattle health.
3. Ruminal pH has been the principal, and, very often in practice, unique criterion to diagnose SARA. However single point rumen sampling lacks of precision and continual reticulorumen pH monitoring can be costly and subject to short electrode lifespan (Hamilton et al., 2019; Jonsson et al., 2019). As rumen hypomotility, or stasis, is a common consequence of a wide range of metabolic diseases (Leek, 1983), the Universities of Glasgow and Strathclyde developed an indwelling sensor to continually monitor reticuloruminal contraction. Therefore, the third objective of this study was to explore the potential of this sensor system and to evaluate the sensor capability to diagnose SARA under experimental conditions.

Chapter 2 Material and Methods

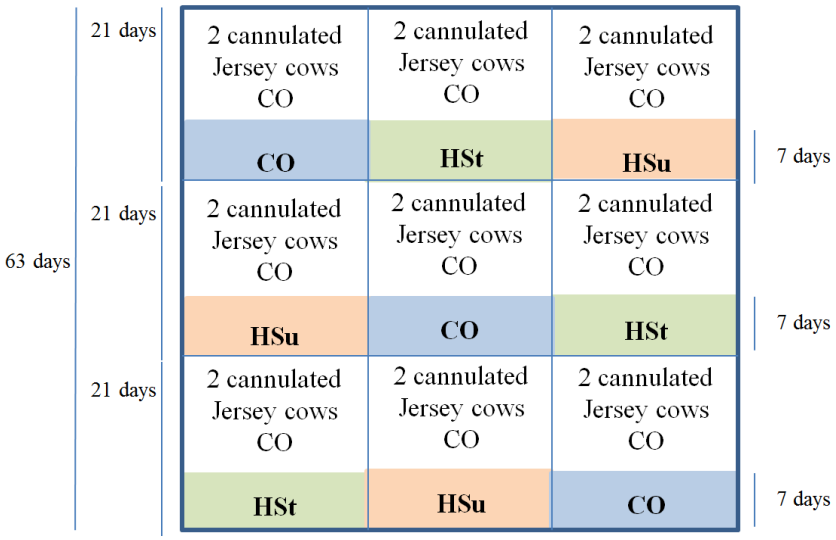
2.1 Study design

The trial involved six adult, non-lactating, rumen-fistulated Jersey cows and was conducted at the University of Glasgow's Cochno Farm near Glasgow, UK. Ethical approval was provided under the Home Office Project Licence PPL 60/4156 (issued 29 January 2013). The mean initial weight for the animals was 625 ± 40 kg (mean ± standard deviation). The study was a 3 × 3 Latin square of nine weeks divided into three periods of three weeks each. During the first two weeks of each experimental period, cows were fed on a maintenance diet (**CON**) composed of 430 g of rolled barley and 10.59 kg of grass silage (DM). In the last week of each experimental period, each animal was allocated to one of three diets: continuation of the existing maintenance diet (CON), a high-starch diet (**HSt**) composed of 5.5 kg of barley and 5.5 kg of silage (DM), and a high-sugar diet (**HSu**) composed of 3.3 kg of barley, 2.2 kg of cane molasses, 20 g of urea and 5.5 kg of silage (DM). The chemical composition of the three diets and their rotation across groups are summarised in Table 2–1 and Figure 2–1 respectively. Dietary components were regularly sampled, analysed by near infrared spectroscopy and results reported according to the Cornell Net Carbohydrate and Protein System to ensure consistency in diet composition. Cows were stabled in pairs with mattresses and wood shavings, and with free access to water. Concentrate was offered individually once daily between 09:00 and 11:00. At 11:00 any left-over was removed, weighed and discharged. Then grass silage was offered at the maximum inclusion rate indicated in Table 2–1. Grass silage was withdrawn at 21:00 in those weeks in which challenge diets were offered or left overnight during the washout periods, in any case without recording the weight. Animals were monitored daily according to four health parameters: faecal consistency, inappetence, depression and ruminal tympany. Faecal consistency was scored using a simplified scale from 0 (not present) to 2 (severe) adapted from Atkinson (2009). Inappetence was scored as 1, if more than 25% of the concentrate was refused, or 2 if it was totally refused. Animals less active compared with pen-mates were scored 1 for depression while animals unable to stand were scored as 2. This last scenario never happened during the trial.

Table 2-1. Diet ingredients and nutrient composition of the three diets fed in the trial

	Control	High-Starch	High-Sugar
Expected Dry Matter intake (kg)	11.02	11.03	11.02
Ingredients, Kg of DM			
Grass Silage (kg DM)	10.59	5.5	5.5
Rolled Barley (kg DM)	0.43	5.53	3.3
Molasses (kg DM)	0	0	2.2
Urea (kg DM)	0	0	0.02
Forage (%DM)	96.1	49.87	49.91
Nutrient composition (% DM)			
Dry Matter%	33.22	47.35	45.72
ME (MJ/kg)	9.96	10.68	10.62
Crude Protein %	14.67	13.1	13.05
Non Fibre Carbohydrate (%DM)	30.52	46.18	48.35
Starch (%DM)	2.78	29.82	17.92
Sugar (%DM)	1.83	2.15	12.64
Lactic acid (%DM)	8.7	4.51	4.92
peNDF (%DM)	36.08	22.48	20.92
Lignin (%DM)	3.72	2.86	2.47
Ash (%DM)	7.61	5.39	8.08

Figure 2-1. Experimental feeding management

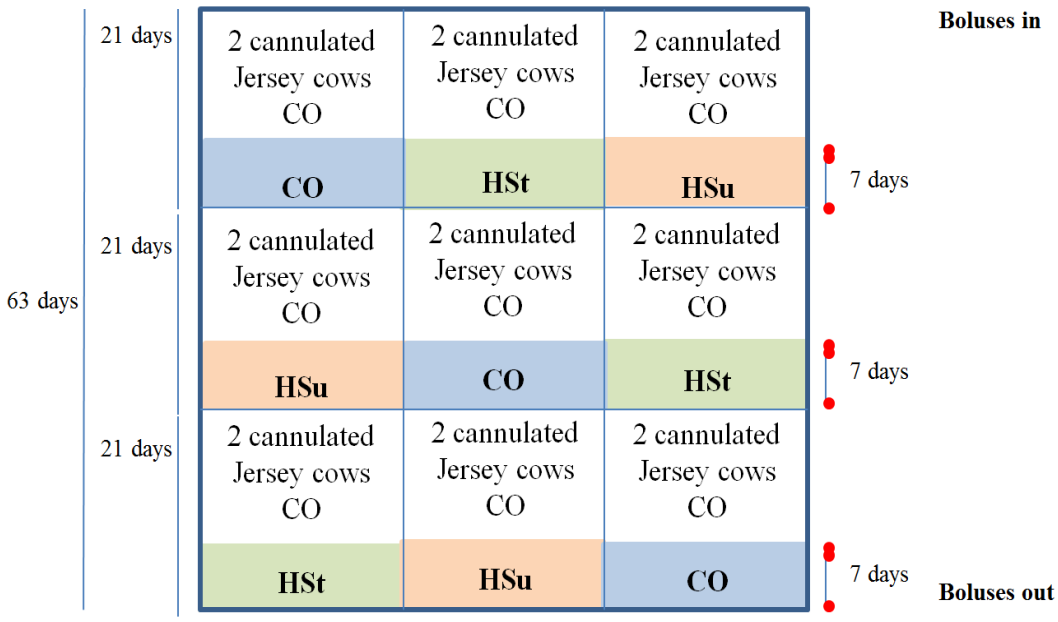


The figure shows the experimental design with the complete timeline and the rotation of the diet across the groups.

2.2 Sample collection and process

All feed samples were stored in the freezer at -20°C until they were analysed. Silage was sampled four times during the trial; barley and molasses two times each. Blood and ruminal samples were taken on the first (**TP-1**), the second (**TP-2**) and the last (**TP-7**) day of each challenge week for a total of nine sampling times. At these time-points the animals had restricted access to feed overnight and the samples were always taken between 08:00 and 10:00, before the diet was delivered. Rumen fluid was collected from the ventral sac of the rumen through the rumen cannula, into two Falcon tubes (15 mL) with and without glycerol (3 mL). Blood was collected via jugular venepuncture into vacutainers containing EDTA, lithium heparin and sodium fluoride and oxalate for haematological analyses, biochemical analyses and glucose determination respectively. All the samples were kept refrigerated till they were processed in the laboratory within four hours. Boluses measuring reticuloruminal pH and motility were inserted the first day. pH boluses were retrieved on the last day of the experiment while, due to the small capacity of the memory card, a new set of motility boluses were used for each period of 21 days.

Figure 2-2. Sampling time during the trial



The figure shows the time points for ruminal and haematological sampling during the trial, as well as the time in which indwelling devices were inserted and retrieved.

2.3 Feed, blood and ruminal samples analysis

Feed analysis was outsourced to an external laboratory (CVAS Dalton, Thirsk, North Yorkshire, YO7 3JA, UK) which analysed silage, barley and molasses using NIR (FOSS 5000 NIR, FOSS UK Ltd, Warrington, WA3 6AE, UK). Haematological and biochemical analyses were performed at the University of Glasgow Veterinary Diagnostic Services laboratory. Table 2–2 reports the inter-assay coefficients of variation and lower detection limits when these were available.

Table 2-2 Coeffients of variation and lower detection limits of the assays used

Assay	Detection Limit	inter assay variation coefficients %
Lymphocytes (10 e9/L)	N/A	2.9
Monocytes (10 e9/L)	N/A	6.9
Neutrophils (10 e9/L)	N/A	1.6
RBC (10 e12/L)	N/A	1.2
PCV (%)	N/A	N/A
SAA (µg/ml)	N/A	N/A
Hp (g/L)	0.02	4.5
NEFA (µmol/L)	0.072	4.5
Glucose (mmol/L)	0.06	11
BHB (mmol/L)	0.1	3.5
GGT (U/L)	7	3
AST (U/L)	5	8
Globulin (g/L)	N/A	N/A
Albumin (g/L)	2	2
Total Protein (g/L)	2	2
Histamine (ng/ml)	3.8	7.3
Propionate (mmol/L)	25	N/A
Butyrate (mmol/L)	25	N/A
Acetate (mmol/L)	25	N/A

2.3.1 Haematology

A flow-cytometer (ADVIA 120, Haematology System, Siemens, UK) was used to obtain haemoglobin, red blood cell count, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, platelet count, white cell count, and differentiated white cell count (neutrophils, lymphocytes, monocytes, eosinophils, basophils).

2.3.2 Biochemistry

An integrated chemistry system (Dimension Xpand Plus, Siemens, UK) was used to obtain sodium, potassium, chloride, calcium, phosphate, magnesium, urea, creatinine, bilirubin, alkaline phosphatase, aspartate aminotransferase (**AST**), gamma-glutamyl transferase (**GGT**), total protein, albumin, globulin, glutamate dehydrogenase (**GLDH**) plasmatic concentrations. Glucose concentration in plasma was determined using a commercial kit (GLUC, Dimension clinical system, Siemens, UK). The method is an adaptation of the hexokinase-glucose-6-phosphate dehydrogenase method described by Kunst and colleagues in 1984 (Kunst, Drager, & Ziegenhorn, 1984). BHB was determined by a commercially available enzymatic method (RANBUT, Randox Laboratories Ltd, UK). The procedure is based on the oxidation of D-3-hydroxybutyrate to acetoacetate by 3-hydroxybutyrate dehydrogenase and the reduction of a cofactor NAD^+ to NADH + H^+. The variance in absorbance determined by the NADH is directly correlated with the BHB concentration. Non-esterified fatty acids (**NEFA**) analysis was outsourced to an external ISO 17025 accredited laboratory (SAC Consulting Veterinary Services, Auchincruive, UK, KA6 5AE).

2.3.3 Serum Amyloid A determination

SAA concentration was determined using a commercially available ELISA kit (SAA-11, Cow serum amyloid A ELISA, Life Diagnostic Inc., UK). The test involved two SAA specific antibodies. SAA is first dissociated from possible interfering factors by heating the sample at 60°C for 1 hour and then, once diluted, incubated in antibody coated microtiter wells together with HRP conjugate for 1 hour. The wells are then washed to remove unbound antibodies, incubated with chromogen reagents and absorbance is measured a 450 nm using FLUOstar OPTIMA plate reader (BMG LABTECH Ltd., Bucks, UK). Results were then analysed and calculated using the MARS (Optima) data analysis software (BMG LABTECH, version 2.40).

2.3.4 Haptoglobin determination

The Hp determination was performed by Institute of Biodiversity Animal Health and Comparative Medicine, University of Glasgow, Bearsden Road, Glasgow, G61 1QH, UK as described by (Eckersall et al., 1999) with minor modifications. Briefly, 3 µL standards and plasma samples were mixed with 150 µL of diluted haemoglobin (60 mg/L Hb, 0.154 M NaCl). At 50 and 75 sec, 135 µL of chromogen and 37.5 µL of hydrogen peroxide (H_2O_2) were added respectively. Absorbance was measured at 5 minutes at a wavelength of 660 nm after H_2O_2 addition using an ABX Pentra 400 automated biochemical analyser (HORIBA UK Ltd., Northampton, UK).

2.3.5 Ethanol and Volatile Fatty Acids determination

Ethanol and VFA concentrations in rumen fluid were determined using high-performance liquid chromatography by an external ISO 17025 accredited laboratory (Sciantec Analytical, Cawood, YO8 3SD, UK). The lab analyser used is an Agilent 7890B Gas Chromatography system (Agilent, Santa Clara, CA 95051, US) with a J&W HP-FFAP GC Column, 30 m, 0.53 mm, 1.00 µm, 5 inch cage (Part Number: 19095F-123E, Agilent, Santa Clara, CA 95051, US).

2.3.6 Histamine determination

The concentration of histamine in ruminal fluid was obtained with a competitive ELISA kit (KA 1888 Histamine ELISA kit, Abnova, Tw). The principle of the test involves the acylation of histamine and the subsequent incubation of unlabelled antibody in the presence of its antigen (within the specimen). These bound antibody/antigen complexes are then added to an antigen coated well. Unbound antibodies are removed by washing. Absorbance is measured within 10 minutes at 450 nm using an ABX Pentra 400 automated biochemical analyser (HORIBA UK Ltd., Northampton, UK).

2.4 Continuous measurement of ruminal pH

The integrated system used to measure rumen pH was originally developed by researchers of Science Park Graz (Stremayrgasse 16, 8010 Graz, At) and then produced by smaXtec, Animal Care Gmbh, (Belgiergasse 3, 8020 Graz, At). The bolus consists in a break-proof synthetic case which lodges the measuring unit, a micro-processor, a lithium-ion battery and a weight. The external dimensions are 132 × 35 mm (length × diameter) and it weighs 220 grams. pH data, animal ID, date and time of the day are all variable that may be added into the integrated system. The measuring unit is activated through contact with a magnet provided by the manufacturers and, following activation, it is submerged in buffer solution (pH 7.00) for calibration. Measurement data are stored in the bolus and automatically transmitted to an external receiver (smaXtec Base Station) by radio transmission (smaXtec, 2019). Data are interpreted and plotted on a graph by the program and raw data may be requested and analysed separately. The bolus measures the pH every 10 minutes, the range of measurement was between pH 3 and pH 9, with an accuracy of ±0.2 up to day 90 following activation and ± 0.4 up to day 150.

In this trial, after initialisation, boluses were inserted through the rumen fistula directly in the reticulum. In 2014 Klevenhusen and co-workers, validated smaXtec boluses as a suitable mean to accurately describe pH variation within the rumen (Klevenhusen et al., 2014).

2.5 Continuous measurement of ruminal motility

Reticuloruminal motility was measured using a prototype motion-sensitive bolus projected and built by a team of engineers of Strathclyde University. Each bolus contained a 3-axis accelerometer with integrated data storage, SD card, real-time clock and a lithium battery with battery life of approximately 30 days. All components were inserted into a cylindrical plastic bolus which was weighted to be at least 200 g to ensure that the device would rest on the floor of the reticulum. The sampling frequency of the accelerometers was configured to be 12.5 Hz. Amplitude of contractions and the inter-contraction periods were identified as described by Hamilton and colleagues (2019).

2.6 Statistical analysis

2.6.1 Health, haematological and biochemical parameters

Health data were obtained for each cow for the first five days of each challenge period. These data were count-based and therefore highly skewed, so were converted to a binary variable for each cow. These presence/absence indicators were then summarised as a 3 × 2 contingency table and analysed using Fisher's exact test to determine the overall effect of treatment group on each binary health indicator. For blood biochemical parameters (continuous observations), linear mixed effects models (**LME**) were applied using treatment and time point as fixed effects, and a random effect of animal ID to control for the repeated observations within animal. Shapiro-Wilk tests and visual appraisal were used to assess the distribution of the residuals for each biochemical parameter, and where these were found to be non-normal (therefore violating the assumption of the LME) the respective data were log-transformed and re-analysed with subsequent re-assessment of normality using the same procedure. All statistical analyses were carried out using R (R Core Team, 2018), with LME models implemented using the lme4 package (Bates et al., 2015).

2.6.2 Reticuloruminal pH and motility

To ensure that we obtained data that reflected the dietary challenge rather than a post-adaptation period, we censored the data to include only those observations from the first five

days after the introduction of each of the three treatments (CON, HSt and HSu). The pH data were analysed using the method described by Denwood et al. (2018) to generate a list of predicted values for each animal according to a generalised additive model fitted with a single sine-wave. For each predicted value, the residual was calculated, and the absolute value of the residual was used for the subsequent analysis. pH data were also analysed using standard descriptive statistics to allow an easier comparison with previous research reports. Numbers of minutes for which pH < 5.6 and pH < 5.8, minimum, maximum and median values were obtained for each cow for each day. Finally, two samples from each of six animals on each of the diets were randomly taken from the pool of all pH values from between 16:00 and 17:00 as described by Jonsson and colleagues (2019) and compared against the thresholds mentioned above. LME were applied using LME4 package (Bates et al., 2015) in R (R Core Team, 2018), in which animal ID was a random factor while treatment and time-points and their interactions were considered as fixed effects. The best model was selected in each case according to the lowest AIC (Akaike information criterion) and BIC (Bayesian information criterion).

Motility readings were initially processed as described by Hamilton and co-workers (2019) to generate a time series of values at 4’30” intervals (270 s) for activity (representing the variance in the energy of the movement vector) and inter-contraction interval (period). To reduce the noise caused by extraneous movements of cattle, a night-only data-frame was established using observations recorded from 21:00 until 07:00.

Chapter 3 Results

3.1 Feed intake and health parameters

The effects of each of the treatments on the health parameters monitored are described in Table 3–1. The high sugar diet was more likely to be partially or completely refused than the control diet ($P < 0.001$), and there was a tendency for the animals on the high starch diet to consume a lower proportion of concentrate than animals offered the control diet ($P = 0.06$). Both the HSt ($P = 0.001$) and HSu ($P = 0.004$) diets were more likely to cause diarrhoea compared with CON diet and this was more likely to happen on the second and fifth day of the challenge ($P < 0.001$). There was no difference between the two challenge diets in the likelihood of diarrhoea. None of the other health parameters differed by treatment or sequence day, as there were very few observations of abnormality. None of the pH indicators (daily median, daily mean absolute residual, minutes per day pH < 5.6 or pH < 5.8) nor either of the motility indicators (amplitude, period) were significantly associated with the probability of refusal of concentrate or with diarrhoea and including pH or motility variables in GLM in which treatment was also included did not substantively improve the model fit in any case (data not shown).

Table 3-1. Effects of treatment on the binary health indicators and feed consumption

Variable	Level	Control	Starch	Sugar	*P*-value
Incomplete feeding	Incomplete	0	5	9	< 0.001
	Complete	30	25	21	
Diarrhoea	Yes	0	9	8	0.005
	No	30	21	22	
Depression	Yes	0	2	0	0.129
	No	30	28	30	
Tympany	Yes	0	0	0	NA
	No	30	30	30	

The contingency table evaluates the effects of treatment on the binary health indicators and feed consumption. Health data were obtained for each cow for the first five days of each challenge period. The *P*-values presented here are the overall *P*-values derived from Chi-square analysis of contingency tables for each.

3.2 Haematology and biochemistry

Haematological, blood biochemical and ruminal biochemical data, with a summary of the effects of diets and time points are shown in Tables 3–2, 3–3 and 3–4. The HSt diet affected several, but not all, the inflammatory markers and some of the VFA. It increased plasma SAA concentration ($P < 0.001$), neutrophil count ($P = 0.0098$), ruminal iso-valerate ($P = 0.0447$) and iso-butyrate ($P = 0.0395$) concentrations, and it decreased lymphocyte count ($P = 0.0144$). The HSt diet also tended to increase ruminal propionate concentration ($P = 0.0967$) and to decrease plasma NEFA concentration ($P = 0.0982$). The HSu diet reduced albumin ($P = 0.003$) and total protein ($P = 0.03$) concentrations in plasma. The mixed-effects model indicated that all the VFA but iso-valerate ($P = 0.074$) increased at TP-2 ($P < 0.05$). The concentration of SAA in plasma ($P = 0.002$) and neutrophils ($P = 0.002$) also increased at TP-2 while lymphocytes decreased ($P = 0.007$). At TP-2 there was a reduction in red blood cell count ($P = 0.045$) and a tendency to reduced PCV ($P = 0.082$) possibly as a consequence of an increase in rumen osmolality. All the effects observed at TP-2 may mainly be linked to an effect of the HSt diet. The concentration of SAA ($P < 0.025$) and all the VFA except acetate ($P = 0.200$) were increased at TP-7 ($P < 0.05$). In contrast, monocytes ($P = 0.014$), RBC ($P = 0.005$), PCV ($P = 0.010$) decreased and GGT ($P = 0.058$) tended to decrease. Both HSt and HSu diets contributed to the effect seen at TP-7, the former for SAA, RBC and PCV, the latter for monocytes and VFA.

Table 3-2. Haematological analytes according to diet fed and time of sampling.

Variable	Sampling time	Treatment									Effect, *P*-value			
		CON			HSt			HSu			Treatment	Treatment	Time	Time
		(median)	Q1	Q3	(median)	Q1	Q3	(median)	Q1	Q3	(HSt)	(HSu)	TP-2	TP-7
Haematological variables														
Lymphocytes (10 e^9/L)	TP-1	2.78	2.47	3.09	2.60	2.35	3.11	2.85	2.55	3.57	0.0144	0.2231	0.0065	0.1571
	TP-2	2.88	2.64	3.31	1.72	1.48	2.36	2.70	1.98	3.39				
	TP-7	2.69	2.46	3.24	2.82	2.28	3.25	2.44	2.31	2.66				
Monocytes (10 e^9/L)	TP-1	0.28	0.25	0.34	0.25	0.23	0.32	0.31	0.19	0.44	0.2120	0.4318	0.1372	0.0139
	TP-2	0.29	0.23	0.32	0.14	0.11	0.16	0.26	0.23	0.35				
	TP-7	0.18	0.15	0.22	0.23	0.17	0.27	0.24	0.21	0.25				
Neutrophils (10 e^9/L)	TP-1	1.41	1.14	1.91	1.50	1.36	1.77	1.35	1.19	1.47	0.0098	0.7514	0.0023	0.7060
	TP-2	1.73	1.57	1.88	3.00	2.74	3.08	1.66	1.42	1.83				
	TP-7	1.31	1.25	1.57	1.37	1.10	1.63	1.81	1.61	1.83				
RBC (10 e^{12}/L)	TP-1	7.05	6.68	7.23	7.31	6.94	7.62	6.93	6.68	7.44	0.9768	0.1519	0.0451	0.0050
	TP-2	6.94	6.82	7.15	6.83	6.70	7.15	6.90	6.44	7.15				
	TP-7	7.02	6.39	7.10	6.82	6.60	6.91	6.69	6.26	7.17				
PCV (%)	TP-1	38.95	37.63	39.98	40.90	39.00	41.70	38.60	35.93	40.90	0.7861	0.2992	0.0822	0.0097
	TP-2	37.70	37.20	40.45	38.55	36.03	40.55	38.20	35.70	39.13				
	TP-7	39.25	38.25	40.03	37.70	35.75	38.75	38.10	36.58	39.40				

RBC: Red blood cell; PCV: Packed cell volume;

The table shows the median concentrations of haematological analytes according to dietary treatment and time of sampling (n = 3). For each median first and third quartiles are also provided. For each analyte a *p*-value for the effect of diet (reference = Control) and time-point (reference = TP-1) is also provided.

Table 3-3. Biochemical serological analytes according to diet fed and time of sampling

Variable	Sampling time	Treatment									Effect, *P*-value			
		CON			HSt			HSu			Treatment (HSt)	Treatment (HSu)	Time TP-2	Time TP-7
		(median)	Q1	Q3	(median)	Q1	Q3	(median)	Q1	Q3				
Concentration in plasma														
SAA (μg/ml)	TP-1	0.08	0.03	0.12	0.10	0.04	0.17	0.11	0.04	0.12	<0.00001	0.1124	0.0021	0.0247
	TP-2	0.14	0.06	0.18	0.50	0.26	0.56	0.13	0.06	0.14				
	TP-7	0.08	0.03	0.09	0.21	0.13	0.27	0.11	0.06	0.17				
Hp (g/L)	TP-1	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.10	0.1987	0.5577	0.9239	0.2850
	TP-2	0.09	0.09	0.10	0.13	0.10	0.17	0.09	0.09	0.10				
	TP-7	0.09	0.09	0.09	0.09	0.09	0.14	0.09	0.09	0.10				
NEFA (μmol/L)	TP-1	234.0	218.0	472.0	314.0	177.5	402.5	316.5	240.7	367.5	0.0982	0.3864	0.1422	0.3363
	TP-2	326.5	261.2	460.0	138.0	114.7	148.5	302.0	195.0	386.5				
	TP-7	245.0	190.7	326.2	364.0	196.5	485.0	242.0	175.0	258.0				
Glucose (mmol/L)	TP-1	3.85	3.65	4.05	4.10	3.58	4.25	3.90	3.90	3.98	0.2632	0.5443	0.7759	0.7628
	TP-2	4.00	3.85	4.23	4.10	3.80	4.18	3.85	3.73	4.05				
	TP-7	3.80	3.65	4.10	4.10	4.03	4.33	3.95	3.75	4.08				
BHB (mmol/L)	TP-1	0.09	0.07	0.11	0.12	0.10	0.14	0.12	0.08	0.16	0.3828	0.7403	1.0000	0.4932
	TP-2	0.10	0.07	0.11	0.15	0.08	0.16	0.05	0.02	0.11				
	TP-7	0.11	0.06	0.10	0.15	0.12	0.20	0.13	0.06	0.16				
GGT (U/L)	TP-1	33.50	29.00	39.50	35.50	32.50	37.75	30.00	28.25	39.25	0.4759	0.4369	0.4894	0.0576
	TP-2	34.00	29.00	38.25	33.50	30.25	37.50	30.50	28.25	34.25				
	TP-7	32.00	27.50	35.75	34.00	31.25	36.75	27.00	25.50	27.00				
AST (U/L)	TP-1	64.00	57.75	65.75	63.00	55.50	69.00	64.50	56.75	75.25	0.7380	0.4194	0.9771	0.6111
	TP-2	65.00	59.25	67.00	63.00	57.50	67.75	62.50	59.50	71.50				
	TP-7	59.00	56.25	62.50	69.50	56.00	74.00	64.50	62.00	69.25				
Globulin (g/L)	TP-1	40.50	37.25	43.75	40.00	38.25	42.50	40.00	36.75	43.25	0.6918	0.1966	0.5338	0.2603
	TP-2	41.50	37.50	44.00	41.50	39.25	43.75	40.00	37.50	41.00				
	TP-7	41.50	39.50	42.75	36.50	32.75	39.50	39.00	35.75	40.00				
Albumin (g/L)	TP-1	37.00	36.00	38.00	37.00	36.25	37.75	36.00	35.00	37.00	0.2322	0.0032	0.6063	0.2322
	TP-2	36.50	36.00	37.75	37.50	37.00	38.00	36.50	36.00	37.00				
	TP-7	37.50	36.25	38.00	35.50	35.00	36.00	35.50	34.25	36.75				
Total Protein (g/L)	TP-1	77.00	74.25	80.50	75.50	75.00	78.25	74.50	73.25	79.50	0.4594	0.0342	0.4594	0.1571
	TP-2	77.00	74.25	82.00	78.50	74.50	81.75	75.00	73.00	77.75				
	TP-7	78.50	76.50	79.75	72.00	70.25	74.50	73.00	72.00	75.50				

SAA: Serum amyloid A; Hp: Haptoglobin; NEFA: non-esterified fatty acid; BHB: β-hydroxybutyrate; GGT: Gamma-glutamyl transferase; AST: aspartate aminotransferase

The table shows the median concentrations of biochemical serological analytes according to dietary treatment and time of sampling (n = 3). For each median first and third quartiles are also provided. For each analyte a *p*-value for the effect of diet (reference = Control) and time-point (reference = TP-1) is also provided.

Table 3-4. Biochemical ruminal analytes according to diet fed and time of sampling

Variable	Sampling time	Treatment									Effect, *P*-value			
		CON			HSt			HSu			Treatment (HSt)	Treatment (HSu)	Time TP-2	Time TP-7
		(median)	Q1	Q3	(median)	Q1	Q3	(median)	Q1	Q3				
Concentration in ruminal fluid														
Histamine (ng/g)	TP-1	0.30	0.24	0.66	1.93	1.37	2.15	0.70	0.61	0.76	0.1516	0.1344	0.8680	0.4437
	TP-2	0.38	0.26	0.70	0.45	0.31	6.92	1.54	1.39	1.65				
	TP-7	1.32	0.71	1.67	0.82	0.55	1.13	2.91	0.91	7.55				
Propionate (mmol/L)	TP-1	6.53	5.89	7.47	8.04	7.13	8.39	8.07	7.25	8.89	0.0967	0.7568	0.0352	0.0023
	TP-2	7.29	6.88	8.62	11.18	10.27	11.94	6.74	6.41	8.87				
	TP-7	9.40	8.26	11.97	8.17	7.07	13.35	9.99	9.25	13.04				
Butyrate (mmol/L)	TP-1	2.91	2.62	3.31	3.80	3.19	4.37	3.73	3.42	3.81	0.0313	0.2875	0.0042	0.0193
	TP-2	3.63	3.36	4.24	6.35	6.00	6.90	3.82	3.71	4.63				
	TP-7	4.26	3.75	5.00	4.43	2.43	6.43	4.90	4.16	5.71				
Acetate (mmol/L)	TP-1	35.44	33.40	38.10	39.03	36.61	42.17	40.02	37.96	41.86	0.4004	0.6749	0.0290	0.1966
	TP-2	38.66	37.53	43.77	50.73	47.55	53.41	36.64	35.55	41.00				
	TP-7	43.65	42.44	46.63	37.33	29.37	45.80	44.65	39.88	47.70				
Iso-Valerate (mmol/L)	TP-1	0.60	0.54	0.69	0.63	0.61	0.67	0.65	0.56	0.68	0.0447	0.4606	0.0738	0.0035
	TP-2	0.55	0.48	0.67	1.01	0.92	1.02	0.66	0.61	0.72				
	TP-7	0.87	0.61	1.07	0.84	0.72	1.00	0.99	0.69	1.15				
Iso-Butyrate (mmol/L)	TP-1	0.50	0.47	0.57	0.50	0.49	0.51	0.51	0.49	0.53	0.0395	0.1929	0.0235	0.0289
	TP-2	0.46	0.44	0.53	0.79	0.70	0.83	0.54	0.54	0.58				
	TP-7	0.59	0.52	0.60	0.58	0.55	0.66	0.77	0.53	0.82				

The table shows the median concentrations of biochemical ruminal analytes according to dietary treatment and time of sampling (n = 3). For each median first and third quartiles are also provided. For each analyte a *p*-value for the effect of diet (reference = Control) and time-point (reference = TP-1) is also provided.

3.3 Ruminal pH

The smaXtec devices generated 56,160 observations during the study period. Data were reduced to include only those observations from the first 5 days after the introduction of each of the three treatments, the dataset obtained included 12,888 observations (CON = 4296, HSt = 4296, Hsu = 4296). The minimum pH was 4.38 and maximum was 7.00. The effect of treatment, sequence day and hour of day on pH, residual pH, time below pH 5.6 and 5.8, were assessed using linear mixed effect models. Hour of day had strong effects on each variable (Figure 3–1, Figure 3–2 and Figure 3–3). The high-starch diet tended (P = 0.056) to and the high-sugar diet reduced (P = 0.010) median pH (-0.064 and -0.088 pH units respectively) and the pH was reduced overall on D-2 (P = 0.004), D-3 (0.008) and D-4 (P = 0.052), with the strength of the effect decreasing from -0.16 to -0.084 pH units over that time. Time (min/d) under pH 5.6 was significantly increased by the HSu (P = 0.001, adding 108 min/d), and a tendency was seen with the HSt treatment (P = 0.096, adding 53.7 min/d). The effect of day was not significant, except for D-3 (P = 0.017, effect = 100 min/d). The amount of time in which pH was less than 5.8 was significantly increased by HSu (P < 0.001, effect = 201.7 min/d) and HSt (P = 0.002, effect = 131.3 min/d) and there was an effect of D-2 (P = 0.0081, adding 147 min/d) and D-3 (P = 0.010, adding 144 min/d). The amount of time that ruminal pH was below each of the thresholds is summarised in Table 3–5 and Figure 3–4. Figure 3–5 shows the variation throughout the day of the absolute mean residuals after fitting the Denwood (2018) model, for each diet, and Figures 3–2 and 3–3 show min/hour for which pH < 5.6 and pH < 5.8 respectively, by diet, over a 24-h period. Absolute mean residual pH was not affected by the sequence-day but it was significantly affected by dietary treatment. Effect sizes were 0.083 for starch (P < 0.001) and 0.063 for sugar (P < 0.001). Table 3–6 shows the pH values randomly sampled during the challenge week for each treatment group, in a simulation of the conventional diagnostic approach, using the method of Jonsson et al., (2019). None of the samples obtained from cows fed the control diet fell below the thresholds of pH 5.8 or 5.6 but 7/12 animals on the HSt diet and 4/12 animals on the HSu diet fell below the higher threshold of pH 5.8. Below pH of 5.6 there were 2/12 animals on the starch diet and 3/12 animals on the sugar diet.

Table 3-5. Time below pH tresholds according diets and sequence day

	Sequence Day	**CON**	**HSt**	**HSu**
pH < 5.8				
	D-1	8.30 (0.00-40.0)	41.7 (0.00-250)	53.3 (0.00-290)
	D-2	0.00 (0.00-0.00)	220 (20.0-460)	323 (0.00-790)
	D-3	0.00 (0.00-0.00)	215 (0.00-500)	322 (50.0-610)
	D-4	0.00 (0.00-0.00)	152 (0.00-700)	193 (0.00-820)
	D-5	41.7 (0.00-250)	78.3 (0.00-170)	167 (0.00-410)
pH < 5.6				
	D-1	0.00 (0.00-0.00)	0.00 (0.00-0.00)	26.7 (0.00-160)
	D-2	0.00 (0.00-0.00)	51.2 (0.00-190)	165 (0.00-560)
	D-3	0.00 (0.00-0.00)	132 (0.00-340)	195 (10.0-530)
	D-4	0.00 (0.00-0.00)	107 (0.00-630)	86.7 (0.00-500)
	D-5	33.0 (0.00-200)	11.7 (0.00-30.0)	100 (0.00-370)

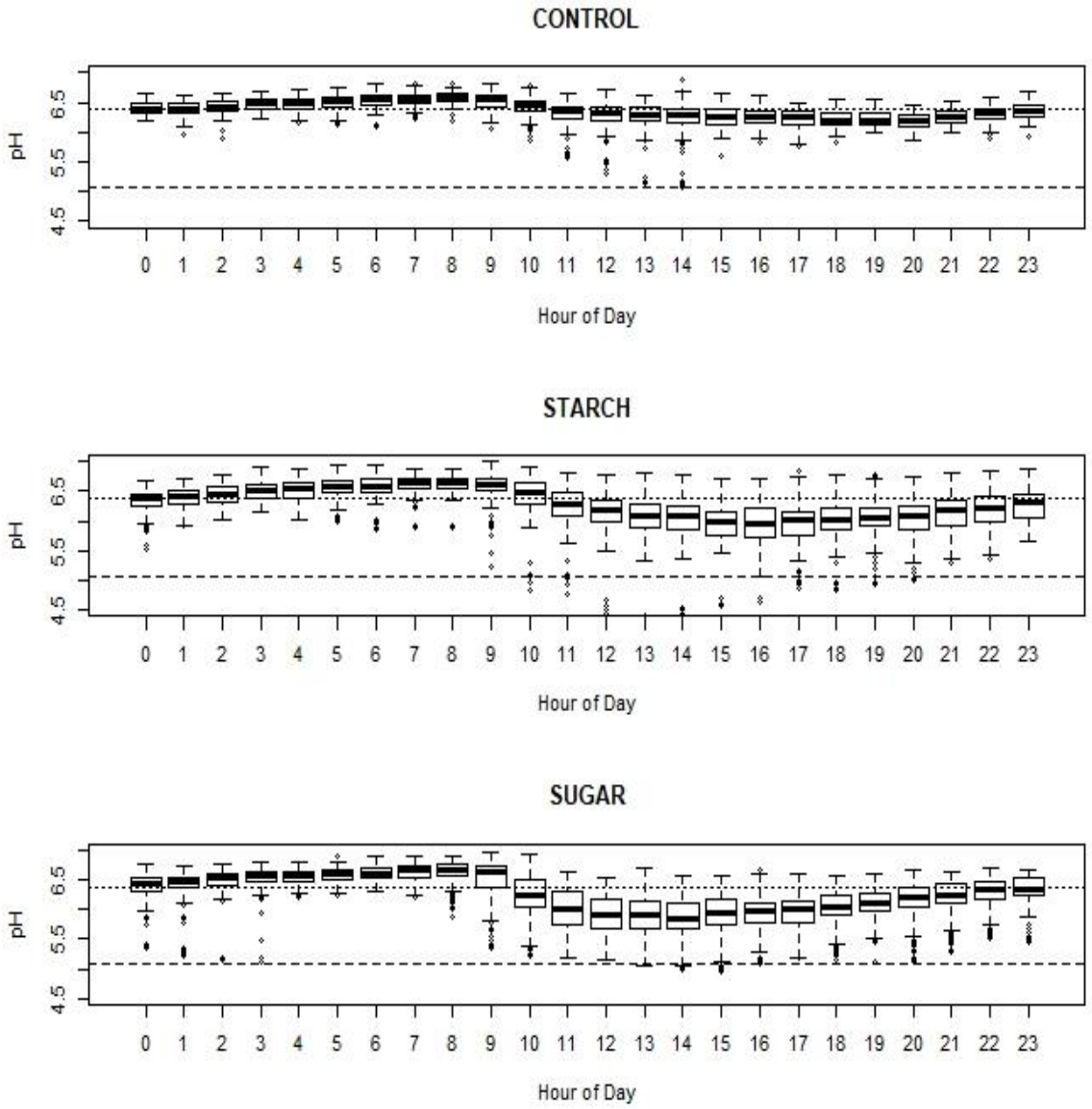
The table shows the amount of time (min/d) in which pH was below thresholds of 5.8 and 5.6 respectively, by sequence day and dietary treatments. Values are expressed as mean (minimum-maximum).

Table 3-6. Single point sampling simulation according to dietary treatment

	Control (pH)	High Starch (pH)	High Sugar (pH)
	6.19	5.05	5.27
	6.31	5.73	5.74
	6.52	5.61	5.54
	6.36	6.71	6.59
	6.26	6.00	6.10
	6.36	5.97	6.21
	6.07	5.69	5.51
	6.21	5.69	5.89
	6.50	6.31	6.07
	6.50	6.16	6.04
	6.23	5.22	6.28
	6.25	5.62	5.91
Total samples pH < 5.8	0/12	7/12	4/12
Total samples pH < 5.6	0/12	2/12	3/12

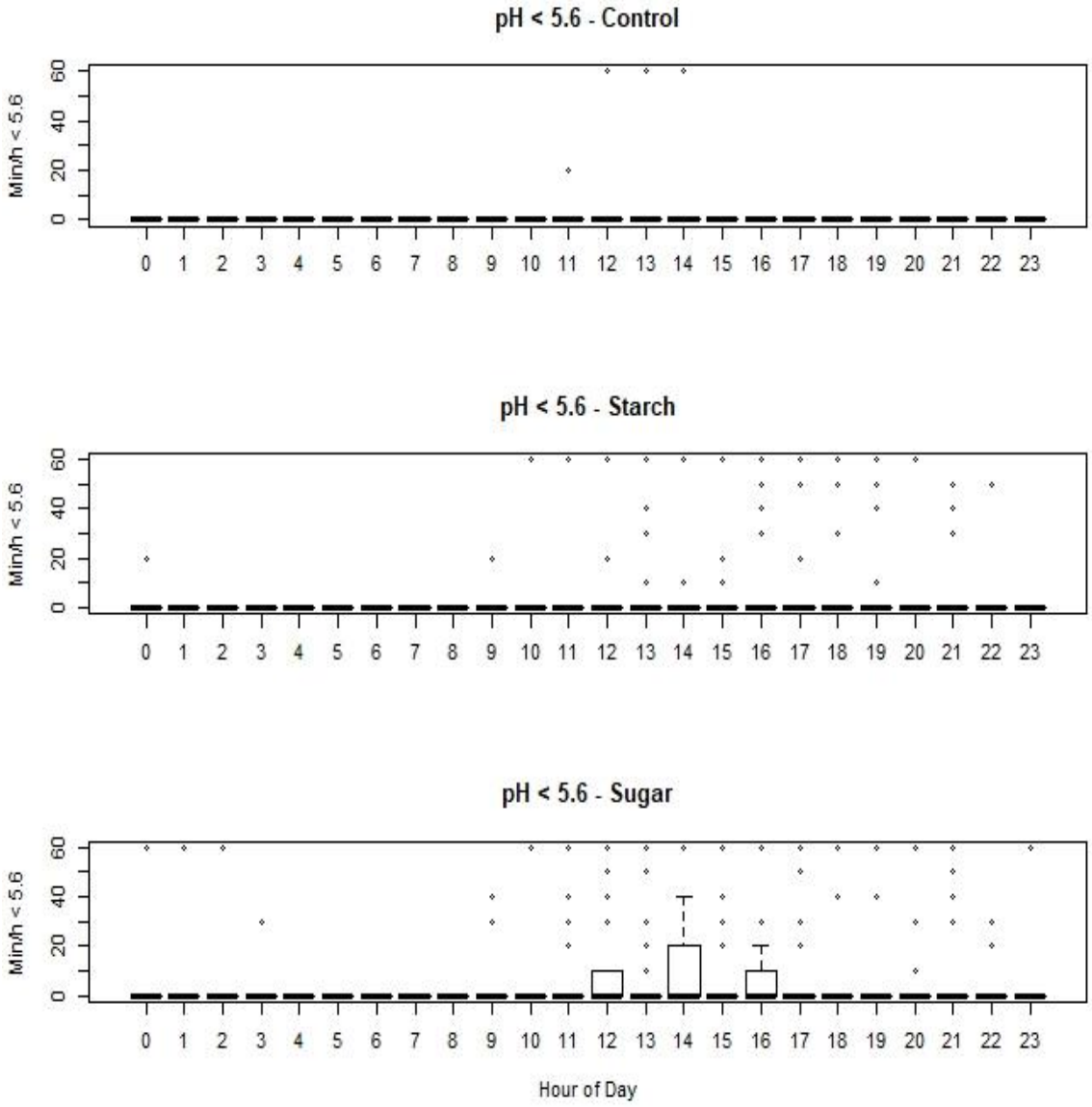
The table shows the results of simulated sampling of reticuloruminal pH. Two samples from each of six animals on each of the diets were randomly taken from the pool of all pH values from between 16:00 and 17:00 as described by Jonsson et al. (2019).

Figure 3-1. pH curves according to diet fed and hour of the day



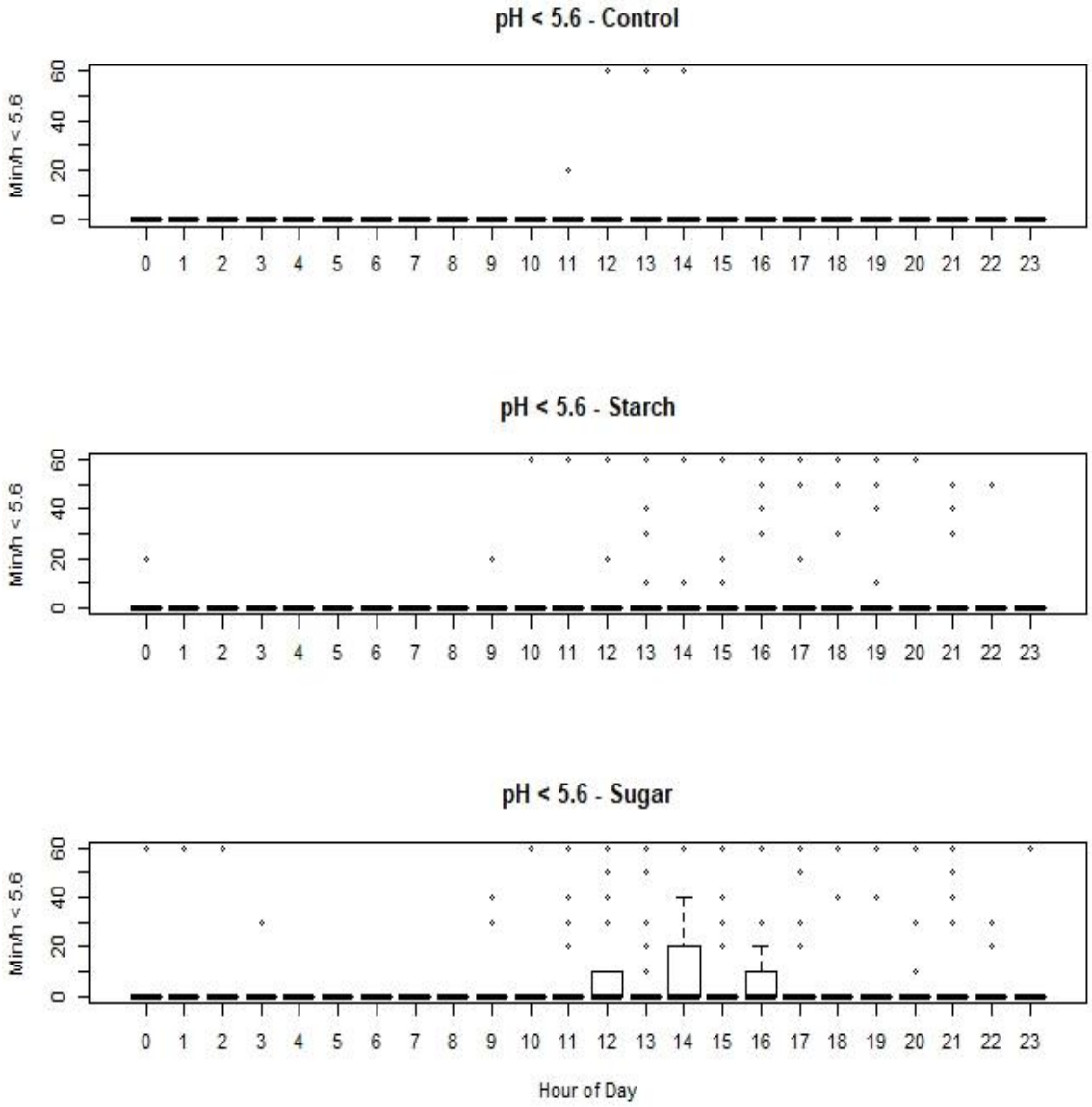
The figure shows the observed pH by hour of day, grouped according to diets (n = 12,888). The horizontal dotted line is the overall median value for the group fed with control diet and the horizontal dashed line is the minimum value for the same group.

Figure 3-2. Time below threshold (pH < 5.6) according to diet fed and hour of the day



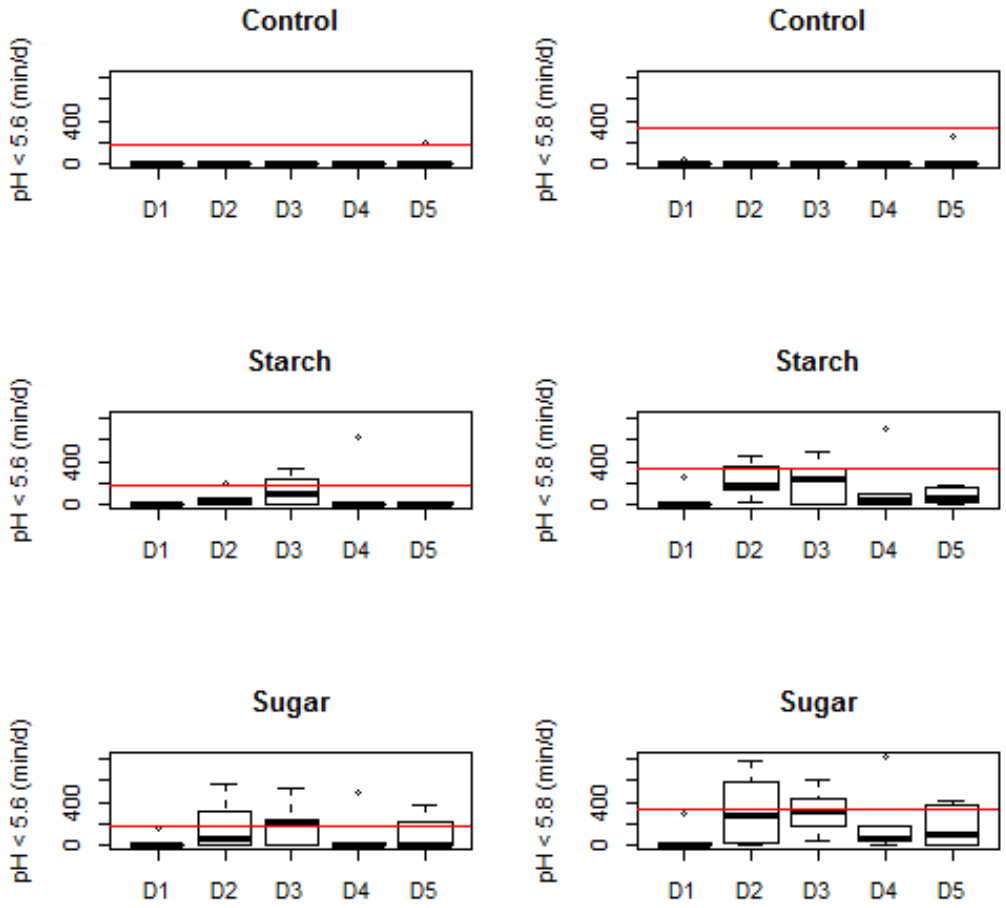
The figure shows the average amount of time (min/hour) in which pH was below 5.6 for each dietary treatment over a 24 hours period (n = 12,888).

Figure 3-3. Time below threshold (pH < 5.8) according to diet fed and hour of the day



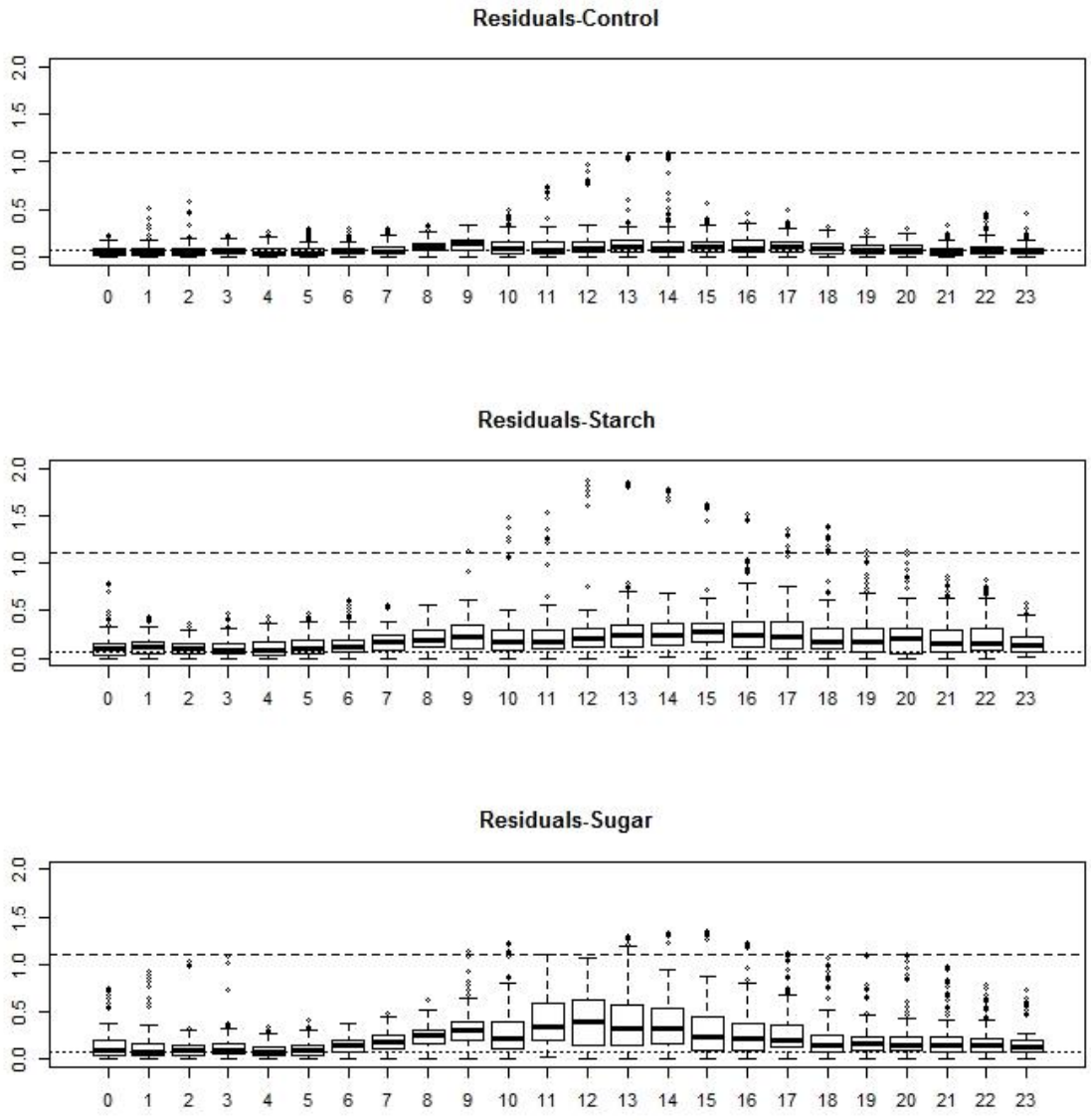
The figure shows the average amount of time (min/hour) in which pH was below 5.8 for each dietary treatment over a 24 hours period (n = 12,888).

Figure 3-4. Time below pH tresholds according diets



The figure shows the amount of time (minutes/day) during which the pH was < 5.6 or 5.8 for each day after each of the treatments (n = 12,888). Threshold values previously defined for SARA (180 min/d at pH < 5.6 and 330 min/d at pH < 5.8) are indicated by a horizontal red line.

Figure 3-5. Distribution of mean absolute residuals by diet fed and hour of day

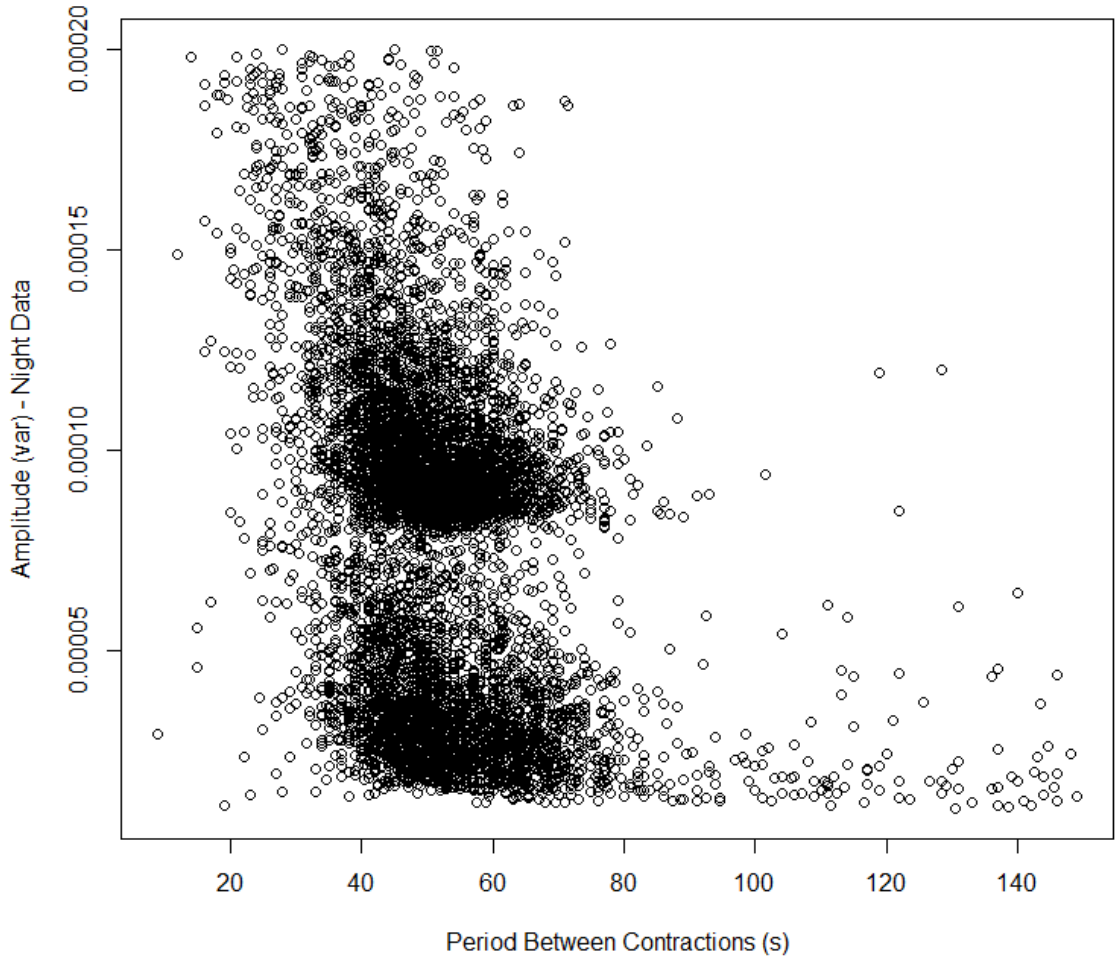


The figure shows the mean absolute residuals by hour of day and dietary treatments (n = 12,888). The horizontal dotted line is the overall median value for the group fed with control diet and the horizontal dashed line is the maximum value for the same group.

3.4 Ruminal motility

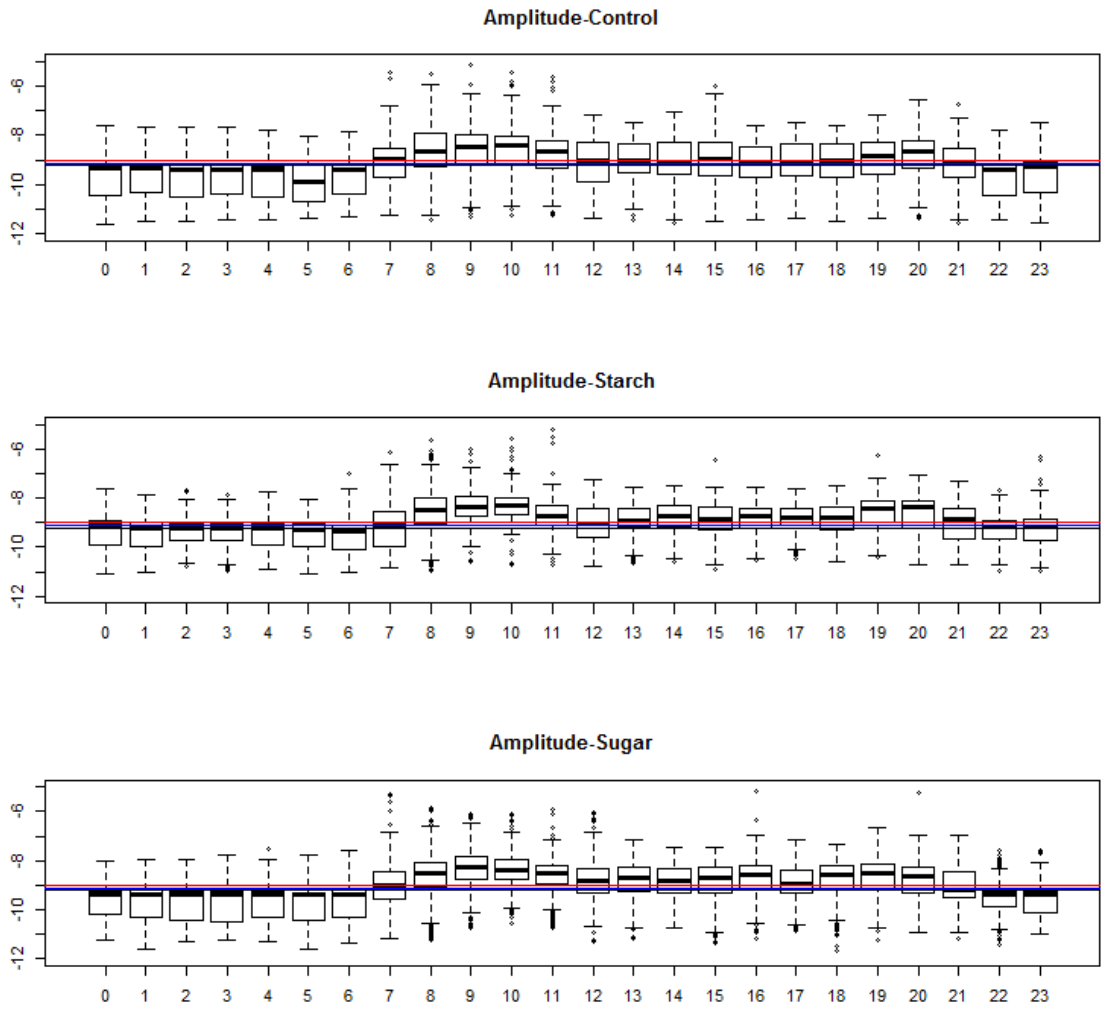
Motility data were incomplete due to irregular blocks of apparent failure to generate a variance signal, resulting in an unbalanced design that limited the potential for analysis of the effects of all factors. In total, 23,883 observations were recorded, with activity (amplitude) ranging from 0 to 0.0104 units and period ranging from 4 to 4,681 s and there were 1,028 observations with periods of > 200 s. Filtering the data to include only those obtained between 21:00 and 07:00 reduced the dataset to 7,607 observations (CON n = 2,961; HSt n = 2,374; HSu n = 2272) and reduced the coefficient of variation from 3.18% to 0.517%. Figure 3–5 shows the overall frequency of nigh-time observations in terms of amplitude and inter-contraction period. The two most densely populated areas in the figure are likely to correspond to the two phases of the reticular contraction. Figure 3–6 is a density plot of the reduced dataset in which the observations are grouped by dietary treatment. It is possible to see how the amplitude of motility recordings was significantly increased by HSt ($P = 0.004$) but not by HSu ($P = 0.161$) diet. The period between presumed contractions was decreased by both starch ($P = 0.002$, effect = -4.16) and sugar ($P < 0.001$, effect = -4.19 s). Figure 3–6 and Figure 3–7 show the effect of hours of the day on amplitude and period respectively. It is possible to notice how, regardless the diets fed, environmental stimuli caused an increased reticular activity (registered as increase in contraction amplitude and decrease in inter-contraction period) at 07:00, probably related to the sight of diets preparation, till 12:00 when most commonly ended of the morning eating bout. Reticular activity increased again between 18:00 and 21:00, probably due to a more intense rumination activity during those hours.

Figure 3-6. Period and amplitude of the motility readings registerd



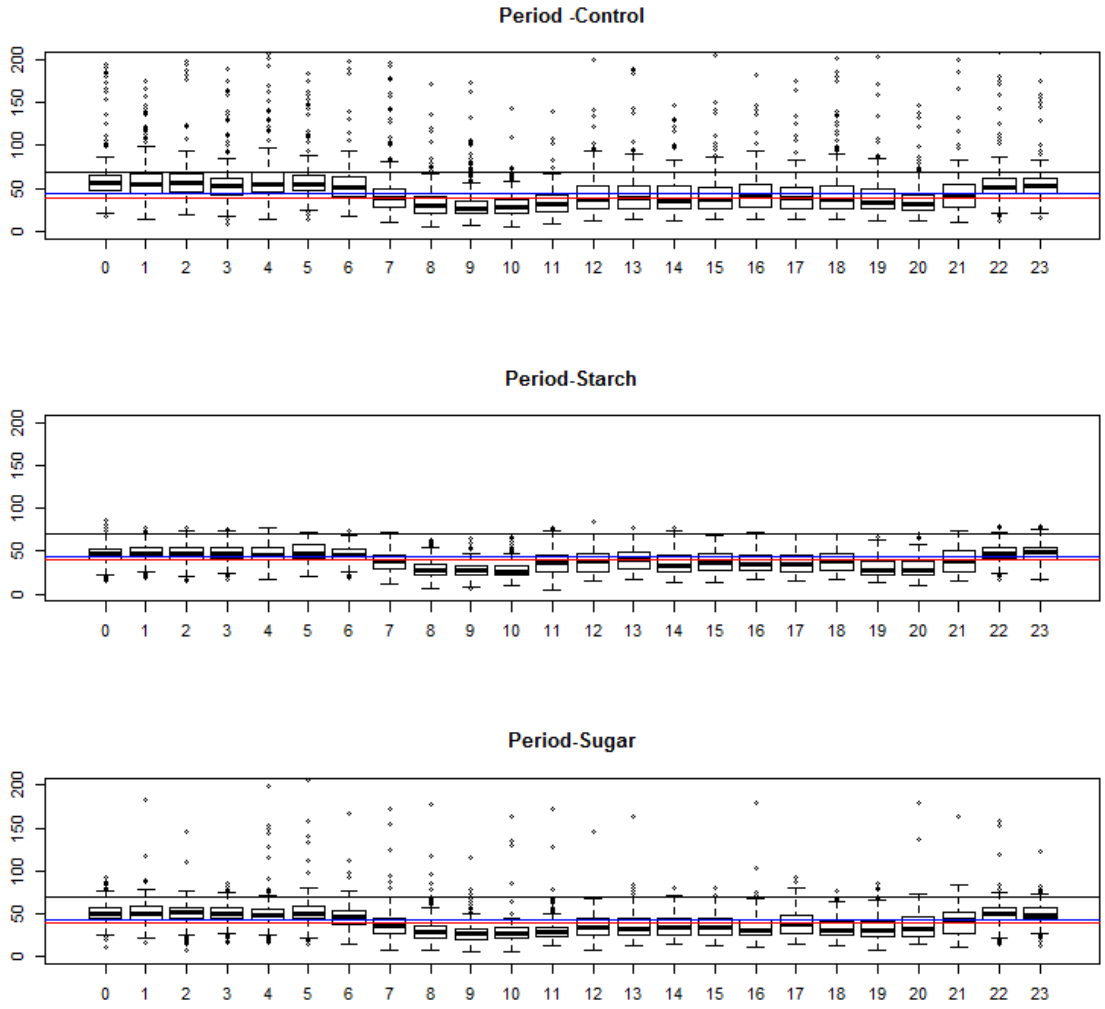
The plot shows the relationship between amplitude and period for observations registered only during the night period (n = 7607). Two distinct sets of readings are recognisable, both with periods of between 40 and 70 s, but separated by amplitude. It is possible that the divergent amplitude signals might represent distinct components of a single reticuloruminal contraction.

Figure 3-7. Daily variation of contractions' amplitude by hour of day and diet fed



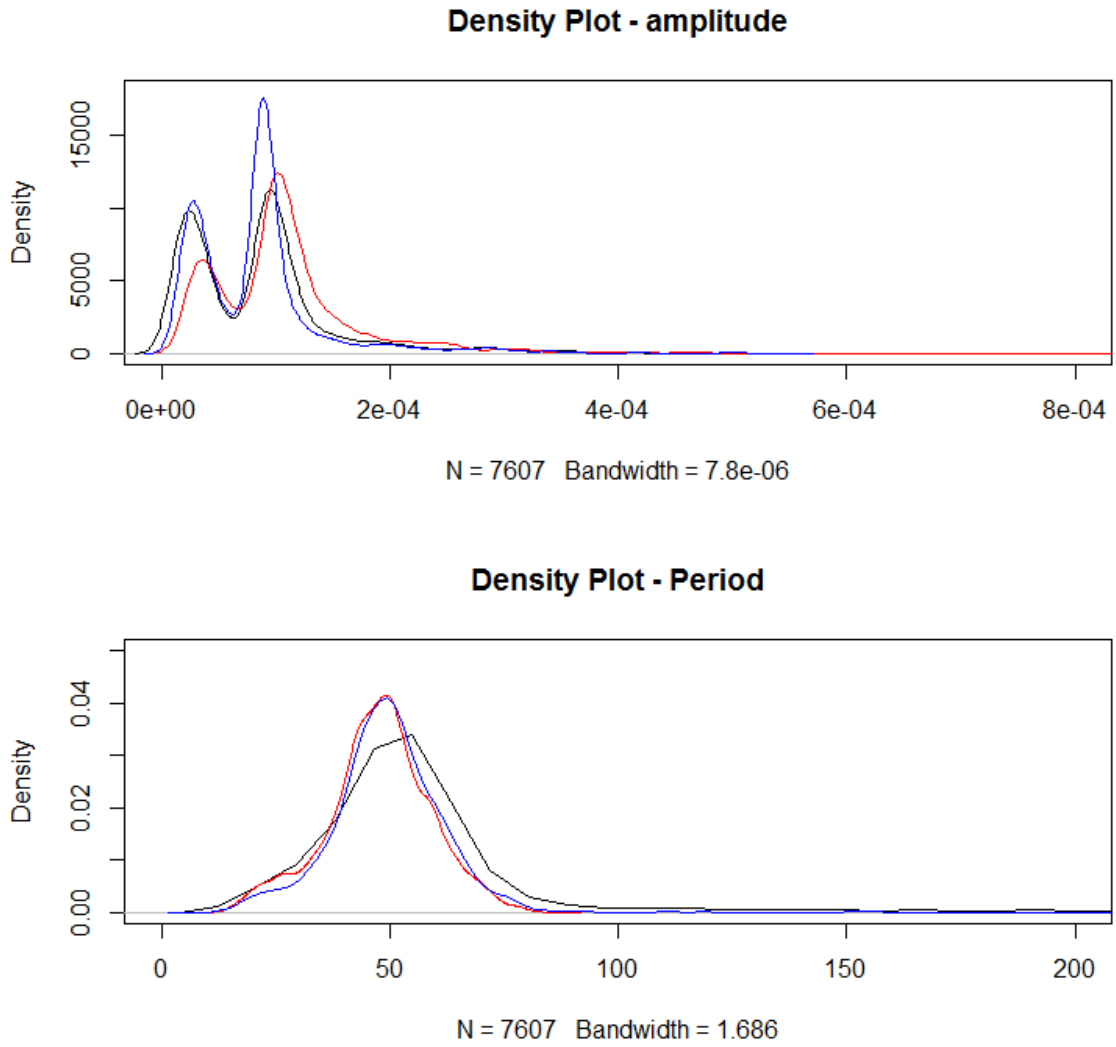
The box and whisker plots show the variation of amplitude of contractions by hour of day, grouped according to treatment (n = 7607). The horizontal black line is the median value for control diet, the red line is the median value for the HSt group and the blue line is the median value for the HSu group.

Figure 3-8. Daily variation of contractions' frequency by hour of day and diet fed



The box and whisker plots show the variation of frequency of contractions (inter-contraction period) by hour of day, grouped according to treatment (n = 7607). The horizontal black line is the median value for control diet, the red line is the median value for the HSt group and the blue line is the median value for the HSu group.

Figure 3-9. Diets effect on reticulorumen motility



The figure represents density plots for motility observations registered only during night time for three treatment groups (n = 7607; black = CO, red = HSt, blue= HSu). HSt but not HSu diet increased amplitude of contractions, while both challenge diets decrease inter-contraction periods.

Chapter 4 Discussion

4.1 Comparison of the systemic effect of the challenge diets

A common model utilised to induce SARA is to reduce the forage:concentrate ratio, usually increasing the starch DM% in the ration, resulting in a rate of VFA production that exceeds the rate of VFA absorption or buffering of protons. We compared two different sources of carbohydrate, hypothesising that this difference would have been reflected in ruminal and overall animal health. Although they belong to the same chemical class of nutrient, rumen metabolism of starches and sugars has been shown to generate different molar proportions of VFA and different sets of secondary metabolites. For instance, sugars tend to increase the molar proportion of butyrate compared with starches and to decrease the molar proportion of propionate and, sometimes, acetate (Martel et al., 2011; Mojtahedi & Danesh Mesgaran, 2011). Using proton nuclear magnetic resonance spectroscopy, gas chromatography-mass spectrometry, and direct flow injection tandem mass spectroscopy Saleem and co-authors investigated the production of secondary metabolites following increasing inclusions of barley in cows' diet. High barley diets have been shown to increase ruminal concentration of a variety of bioactive compounds such as putrescine, methylamines, N-nitrosodimethylamine or ethanolamine, which have the potential, upon absorption, to initiate a systemic inflammatory process (Saleem et al., 2012). Although molasses metabolome has not been characterised yet, several bioactive molecules have been identified in the raw product, and it is well recognised that, with the relative abundance of sugars as substrate, decarboxylation of amino-acids may drift toward deamination, generating potentially harmful biogenic amines (Sanford, 1963; Wang et al., 2013; Ali et al., 2019). We described the effects of two different, iso-energetic and iso-nitrogenous SARA-inducing diets. F:C was 50:50 for both diets, starch and sugar content of the HSt diet were 30% and 2% (DM basis) and 18% and 12.5% in the Hsu diet (DM basis). The reflection of challenge diets on animals' clinical parameters was as expected. Both the HSt and HSu diets increased the probability to have diarrhoea compared with the CON diet but there was not a detectable difference between the two challenge diets. HSu diet only was associated with a reduced probability for an animal to completely eat its concentrate allocation, and this was not expected as in several studies inclusion of sugar increased or tended to increase DMI however none of these trials had such a high inclusion of sugar as ours. (DeFrain et al., 2004; Broderick et al., 2008; Penner & Oba, 2009; Gao & Oba, 2016).

The potential inflammatory effects of diet were assessed using haematology and plasma concentrations of SAA and Hp. In our study, the HSt diet caused an increase in SAA concentrations in plasma, ranging from 0.01 µg/mL to 0.58 µg/mL, whereas the HSu diet had no significant effect. This result appears to be mild in comparison with previous reports. For instance SAA increased up to 170 µg/mL, 430 µg/mL and 498 µg/mL in three trials (Gozho et al., 2005; Gozho, Krause, & Plaizier, 2007; Khafipour, Krause, & Plaizier, 2009a). However, it has to be mentioned that in all the studies cited, the diets reflected much more severely on ruminal pH compared to ours. In particular the average time spent below pH 5.6 and 6.0 was 134 min/d and 404 min/d in the first, 309 min/d and 741 min/d in the second and 279 min/d and 678 min/d in the last study considered. In our study, the overall treatment mean values for both challenge diets were much lower and SARA thresholds were met by some cows only in some days (see Table 3–5). Horadagoda and co-authors (1999) suggested that plasma concentrations < 8.8 mg/L should be considered to be within the reference range for SAA. However, despite the relatively low values we registered, the increase in concentration detected was associated with a significant increase in the count of circulating neutrophils and a concomitant decrease in the count of circulating lymphocytes. Neither diet caused a detectable increase in plasma Hp concentration, which might be due to the lower sensitivity of Hp in detecting inflammatory process (Horadagoda et al., 1999). Collectively, we consider these observations consistent with the presence of a mild, acute inflammatory response triggered by the HSt diet. In contrast, CON and HSu diets did not have any detectable effect on any of the inflammatory markers considered, despite the severe changes in pH registered with the latter.

According to our knowledge, this is the first time that the inflammatory response subsequent to a high sugar challenge has been characterised and compared with the response that follows a high starch challenge. Since no difference in reticuloruminal histamine concentration was attributable to diet, the contribution of LPS might be hypothesised. However, it has been previously demonstrated that low ruminal pH and increased ruminal concentrations of LPS are not sufficient conditions to cause inflammation, but it is necessary for LPS to first translocate into the bloodstream (Khafipour, Krause, & Plaizier, 2009a, 2009b). The translocation site is still a matter of debate as the stratified ruminal epithelium might be less susceptible to mechanical or chemical damage than, for instance, large intestinal monolayer epithelium (Graham & Simmons, 2005; Plaizier et al., 2018). Moreover, as stated above, high barley diets have been shown to increase ruminal concentrations of many bioactive compounds and therefore we cannot confidently state which xenobiotics, or combination of

xenobiotics, activated the inflammatory response and in which site of the digestive tract the xenobiotic absorption happened. We observed, during those weeks when challenge diets were offered, a decline in ruminal pH associated with both HSt and HSu diet, but HSu diet resulted in a longer time in which the reticuloruminal pH fell below thresholds. On average, HSu diet was associated with 212 min/d spent below pH 5.8 and 115 min/day spent below pH 5.6, compared with 141.5 min/d below pH 5.8 and 60 min/d below pH 5.6 on the HSt diet. Sugars are generally more rapidly fermented in the rumen, hence it is logical to assume that increasing the inclusion rate of sugars in the diet should lead to a greater ruminal accumulation of VFA compared with starches (Lanzas et al., 2007). However inclusion of various sugars in the diet have not always led to reductions in ruminal pH values (Oba, 2011). Instead, the inclusion at different rates of different sugar sources did not impact on ruminal pH in some trials, while it increased average pH or decreased the amount of time spent below thresholds in some others (Broderick & Radloff, 2004; DeFrain et al., 2004; Broderick et al., 2008; Chibisa et al., 2015). However, many factors could explain the differences observed, such as variability of sampling time and sampling technique across studies, physical presentation of the ration and metabolic status (lactating vs non-lactating) of the animals. It may therefore be inappropriate to draw any definitive conclusion on the effect of sugar inclusion or rumen pH dynamics until more studies are available for comparison. We concluded that HSu diet was associated with a higher probability of feed refusal and longer time in which ruminal pH was below defined thresholds, while HSt diet was associated with subclinical signs of systemic inflammation.

4.2 Efficacy of recent descriptive ruminal pH models applied to SARA diagnosis

SARA is mostly described as a metabolic disorder characterised by repeated bouts of depressed ruminal pH (Gozho et al., 2005). Different thresholds have been discussed in the past according to the technique used to sample rumen fluid and the rumen compartment where the samples were taken (Duffield et al., 2004; Neubauer et al., 2018). We selected pH < 5.6 and pH < 5.8 as critical thresholds to define SARA because both are widely reported in the literature and we kept them constant throughout our analysis (Gozho et al., 2005; Khafipour, Krause, & Plaizier, 2009a, 2009b; Li et al., 2012; Wang et al., 2013; Chibisa et al., 2015). Within these values, we applied three different models to analyse ruminal pH dataset. In the first model we measured how much time each animal spent below the selected threshold for each challenge day. This concept is not new, having been applied several times previously, and it requires the establishment of a second critical threshold of time. Gozho and co-authors (2005) suggested that 180 min/d spent below pH 5.6 were necessary to experience SARA, while Zebeli et al. (2008) stated that cows may not experience SARA if time spent below pH 5.8 is shorter than 330 min/d. Applying these criteria, the conditions for SARA were never met by the control group, and, they were met by the group fed with HSu diet, on day 3, only with the threshold of pH 5.6. The overall treatment mean values on any other combination of day and threshold did not meet the SARA definition adopted, but, nonetheless, several cows fed HSt exceeded these thresholds between days 2-4, and several cows fed HSu diet did it between days 2-5.

Another approach to diagnose SARA is the collection of samples from twelve animals, of which three should have a pH below 5.5 (Garrett et al., 1999). Without modifying the above mentioned cut-off values we simulated this sample collection using the method described by Jonsson et al. (2019). None of the samples obtained from cows fed CON diet fell below the thresholds during these periods, whereas 7/12 animals (HSt) and 4/12 animals (HSu), and 2/12 animals (HSt) and 3/12 animals (HSu) fell below the thresholds of pH 5.8 and 5.6 respectively. Therefore, in this simulation, both diets would have resulted triggering SARA on the higher threshold, while only the group fed with HSu diet would have been considered at risk for SARA if the cut-off values considered was 5.6.

The third approach, based on the work of Denwood and colleagues (2018), hypothesised that the absolute mean residual of pH, after fitting a model that accounted for an individual

animal's diurnal rhythm and median pH, would be closely related with an animals' health status. In brief, the model creates the best fit pH curve based on a generalised additive model with a superimposed sine-wave for diurnal variation, animal historical data and then calculates, for each pH value recorded, the absolute deviation from the predicted curve. Due to its self-referential nature, this model does not require any pre-selected threshold as the best fit curve is unique for every animal.

All the three models indicated HSu diet as the diet with the higher impact in term of ruminal pH. Cows fed this diet had the highest absolute mean residual, spent the longest time below thresholds 5.6 and 5.8, and had a higher probability to be considered at risk for SARA using threshold 5.6 in a single time point measurement. These results are in partial contrast with those obtained analysing the systemic effect of the diet upon animals' health. HSu diet had a higher rate of feed refusal compared HSt diet, but no difference was detected in terms of diarrhoea occurrence. Moreover, despite the apparently milder effect on rumen milieu, HSt (but not HSu) diet was characterised by the presence of a systemic inflammatory response. These results are in agreement with those published by Khafipour and colleagues (2009a, 2009b). The authors compared a diet with lower F:C with a second one with lower peNDF. The diet lower in peNDF resulted in much more severe ruminal pH conditions without increasing plasma concentration of APP.

Taken together these observations are consistent with a diagnosis of SARA with both challenge diets and therefore we contend that the experimental challenge to induce SARA was effective. None of the models applied resulted in better predictions of the presence of systemic inflammatory response and this is suggestive that reticuloruminal pH is a clinical parameter that should not be used alone to determine the presence or the absence of the disease.

4.3 Evaluation of the capability of newly developed sensor system to diagnose SARA under experimental condition

Ruminal motility is under the control of both extrinsic and intrinsic pathways. Intrinsic innervation has been described only after experimental vagotomy, as the presence of ruminal motility characterised by not being subject to the same inhibitory or excitatory factors of the normal contractions and not presenting an organised functional pattern (Constable, Hoffsis, & Rings, 1990a). Extrinsic innervation consists of gastric centres, located in the medulla oblongata, the vagus nerves, which connect the forestomach with the gastric centres and provides parasympathetic control, and splanchnic nerves originating from the thoracolumbar segment, which provide sympathetic, mainly inhibitory, inputs. The gastric centres do not appear to have a spontaneous rhythm, therefore, in order to periodically originate a depolarization wave, are dependent upon excitatory afferent inputs (Leek, 1969). Excitatory signals are generated by mechanoreceptors located in the oral mucosa, low threshold tension receptors, most densely located in the medial wall of the reticulum and chemical receptors that perceive acidification of abomasal content (Leek, 1969; Gregory, 1987). All these receptors stimulate the gastric centres which, in turn, enhance strength and rhythm of reticulorumen contraction via vagal nerves. In contrast, high threshold tension receptors and acid-sensitive receptors in the reticulum, tension receptors in the abomasum, pain and pyrexia downregulate the gastric centres, and hence, reticuloruminal contraction (Constable, Hoffsis, & Rings, 1990a).

Motility of the forestomach has been investigated in the past to describe pathophysiological changes that occur following acute lactic acidosis, however, to the author's knowledge, this is the first time that this concept is applied to SARA diagnosis and that motility has been monitored over a period of several days. In this study we continuously monitored ruminal contraction, inter-contraction period and variance in amplitude of the contraction with a prototype motion-sensitive bolus which contained three axis accelerometer configured to sample the X, Y and Z acceleration at 12.5 Hz.

In our study, amplitude was highest and most variable during day-time and lowest and most consistent during the night, while period of the contractions followed the inverse pattern, being shorter during the day. The overall increased activity in the reticulorumen is likely to be the results of environmental external stimuli. In fact both amplitude and period of contractions start to move away from night basal pattern between 07:00 and 08:00 and the

period of increased activity lasted till 12:00. This is the time when farm operations such as stockman entering the shed, feed preparation and delivery and eating happened. The contraction rate seems to slow down across midday (13:00 – 17:00) and to increase again between 18:00 and 21:00. It possible to hypothesise that animals had periods of intensive rumination after the morning feeding and a second eating bout in the early evening. Indeed rumination rate has been reported to increase from an average, at rest, of 60 cycles/hour up to 105 cycles/hour during feeding, and to decrease to 50 cycles/hour during rumination periods (Sellers & Stevens, 1966; Constable, Hoffsis, & Rings, 1990a). However to the author's knowledge, the effect of a basal circadian pattern or reticulorumen motility has not been investigated, as it has been done for ruminal pH, therefore a contribution of this factor cannot be excluded (Kimura et al., 2012; Antanaitis et al., 2016; Denwood et al., 2018).

The prototype we used in our experiment was initially evaluated by Hamilton and colleagues (2019) and in this study, we tested the performance of the boluses in a clinical scenario in which mild episodes of rumen acidosis were induced over a relatively long time of period. Indeed, in the past, the main focus with regards to rumen motility has been to identify clinical parameters that abolish rumen function. For instance Crichlow and Chaplin (1985) caused an acute grain overload in fistulated sheep placing ground wheat (40 g/kg BW) and water in a 50:50 ratio through the rumen cannula. They monitored ruminal contraction amplitude and frequency every 2 hours for 12 hours after the engorgement (i.e. when all the nine animals developed rumen stasis). At the first check, 2 hours after the engorgement, amplitude and frequency increased in all the animals but decreased in the following 10 hours until the complete abolition of reticulorumen motility. In our study we selected the observations recorded during night time, as they were characterised by a lower degree of variation, and summarised their effect on contraction amplitude over seven days and we found that HSt but not HSu diet increased the amplitude of rumen contraction. The HSt diet led to a more persistent increase of VFA in the rumen (i.e. VFA detected 24 hours after feed delivery; Table 3–4) and it might be hypothesised that VFA might exert not just an inhibitory, but, to a certain extent, also an excitatory effect on the gastric centres. The period between movements was decreased by both diets and this seems to reinforce the hypothesis that the gastric centres has different circuits that control rate and amplitude of the contraction (Gregory, 1987). In conclusion, the two diets we fed had differential effects on reticuloruminal motility and the boluses were sensitive enough to detect this variation. However further studies to monitor reticuloruminal contractions in the long term are necessary to characterise the normal diurnal pattern of reticulorumen contraction, to verify

if circadian rhythm has an influence on reticulorumen motility and to relate the reticulorumen motility pattern to the overall cow health status.

Conclusion

In conclusion we demonstrated that diets with equal F:C resulted in similar ruminal pH dynamics but different sources of carbohydrate reflected differently on animal health as detected from several acute phase response markers. We tested different models to interpret continual pH records in their ability to predict or detect SARA and none of them fully reflected haematological and biochemical results or, simply, performed better than others. In the end we tested a prototype of indwelling reticular motility bolus as a diagnostic tool for monitoring cow health and diagnose SARA with encouraging results. However it will be necessary a better characterization of normal diurnal pattern of reticulorumen motility before these devices may be used for clinical or research purposes.

Bibliography

Adams, A.E., Olea-Popelka, F.J., & Roman-Muniz, I.N., 2013. Using temperature-sensing reticular boluses to aid in the detection of production diseases in dairy cows. *J. Dairy Sci.* 96, 1549–55.

Ali, S.E., El Gedaily, R.A., Mocan, A., Farag, M.A., & El-Seedi, H.R., 2019. Profiling metabolites and biological activities of sugarcane (Saccharum officinarum Linn.) juice and its product molasses via a multiplex metabolomics approach. *Molecules* 24, E934.

Allen, M.S., 2000. Effects of Diet on Short-Term Regulation of Feed Intake by Lactating Dairy Cattle. *J. Dairy Sci.* 83, 1598–1624.

Allen, M.S., 1991. Carbohydrate nutrition. *Vet. Clin. North Am. Food Anim. Pract.* 7, 327–40.

Alsaaod, M., Fadul, M., & Steiner, A., 2019. Automatic lameness detection in cattle. *Vet. J.* 246, 35–44.

AlZahal, O., Kebreab, E., France, J., Froetschel, M., & McBride, B.W., 2008. Ruminal temperature may aid in the detection of subacute ruminal acidosis. *J. Dairy Sci.* 91, 202–7.

Ambriz-Vilchis, V., Jessop, N.S., Fawcett, R.H., Shaw, D.J., & Macrae, A.I., 2015. Comparison of rumination activity measured using rumination collars against direct visual observations and analysis of video recordings of dairy cows in commercial farm environments. *J. Dairy Sci.* 98, 1750–1758.

Antanaitis, R., Zilaitis, V., Juozaitiene, V., Stoškus, R., & Televičius, M., 2016. Changes in reticulorumen content temperature and pH according to time of day and yearly seasons. *Pol. J. Vet. Sci.* 19, 771–776.

Aschenbach, J.R., Penner, G.B., Stumpff, F., & Gäbel, G., 2011. Ruminant Nutrition Symposium: Role of fermentation acid absorption in the regulation of ruminal pH. *J. Anim. Sci.* 89, 1092–107.

Atkinson, O., 2014. Prevalence of subacute ruminal acidosis (SARA) on UK dairy farms. *Cattle Pract.* 22, 1–9.

Atkinson, O., 2009. Guide to the rumen health visit. *In Pract.* 31, 314–325.

Baldwin, R.L., & Allison, M.J., 1983. Rumen metabolism. *J. Anim. Sci.* 57 Suppl 2, 461–77.

Bates, D., Maechler, M., Bolker, B., & Walker, S., 2015. Fitting linear mixed-effect models using lme4. *J. Stat. Softw.* 67, 1–48.

Bradford, B.J., Yuan, K., & Ylioja, C., 2016. Managing complexity: Dealing with systemic

crosstalk in bovine physiology. *J. Dairy Sci.* 99, 4983–4996.

Bristow, D.J., & Holmes, D.S., 2007. Cortisol levels and anxiety-related behaviors in cattle. *Physiol. Behav.* 90, 626–8.

Broderick, G.A., Luchini, N.D., Reynal, S.M., Varga, G.A., & Ishler, V.A., 2008. Effect on production of replacing dietary starch with sucrose in lactating dairy cows. *J. Dairy Sci.* 91, 4801–4810.

Broderick, G.A.A., & Radloff, W.J.J., 2004. Effect of molasses supplementation on the production of lactating dairy cows fed diets based on alfalfa and corn silage. *J. Dairy Sci.* 87, 2997–3009.

Chamberlain, D.G., Thomas, P.C., Wilson, W., Newbold, C.J., & Macdonald, J.C., 1985. The effects of carbohydrate supplements on ruminal concentrations of ammonia in animals given diets of grass silage. *J. Agric. Sci.* 104, 331.

Chibisa, G.E., Gorka, P., Penner, G.B., Berthiaume, R., & Mutsvangwa, T., 2015. Effects of partial replacement of dietary starch from barley or corn with lactose on ruminal function, short-chain fatty acid absorption, nitrogen utilization, and production performance of dairy cows. *J. Dairy Sci.* 98, 2627–40.

Constable, P.D., Hoffsis, G.F., & Rings, M.D., 1990a. The reticulorumen: normal and abnormal motor function. Part I. Primary contraction cycle. *Compend. Contin. Educ. Pract. Vet.* 12, 1008–1014.

Constable, P.D., Hoffsis, G.F., & Rings, M.D., 1990b. The reticulorumen: Normal and abnormal motor function. Part II. Secondary contraction cycles, ruminaion and esophageal groove closure. *Compend. Contin. Educ. Pract. Vet.* 12, 1169–1174.

Crichlow, E.C., & Chaplin, R.K., 1985. Ruminal lactic acidosis: relationship of forestomach motility to nondissociated volatile fatty acids levels. *Am. J. Vet. Res.* 46, 1908–11.

DeFrain, J.M., Hippen, A.R., Kalscheur, K.F., & Schingoethe, D.J., 2004. Feeding lactose increases ruminal butyrate and plasma β-hydroxybutyrate in lactating dairy cows. *J. Dairy Sci.* 87, 2486–2494.

Denwood, M.J., Kleen, J.L., Jensen, D.B., & Jonsson, N.N., 2018. Describing temporal variation in reticuloruminal pH using continuous monitoring data. *J. Dairy Sci.* 101, 233–245.

do Nascimento, C.O., Hunter, L., & Trayhurn, P., 2004. Regulation of haptoglobin gene expression in 3T3-L1 adipocytes by cytokines, catecholamines, and PPARgamma. *Biochem. Biophys. Res. Commun.* 313, 702–8.

Dong, G., Qiu, M., Ao, C., Zhou, J., Khas-Erdene, Wang, X., Zhang, Z., & Yang, Y., 2014. Feeding a high-concentrate corn straw diet induced epigenetic alterations in the

mammary tissue of dairy cows. *PLoS One* 9, e107659.

Drackley, J.K., 1999. ADSA Foundation Scholar Award. Biology of dairy cows during the transition period: the final frontier? *J. Dairy Sci.* 82, 2259–73.

Drackley, J.K., & Cardoso, F.C., 2014. Prepartum and postpartum nutritional management to optimize fertility in high-yielding dairy cows in confined TMR systems. *animal* 8, 5–14.

Duffield, T., Plaizier, J.C., Fairfield, A., Bagg, R., Vessie, G., Dick, P., Wilson, J., Aramini, J., & McBride, B., 2004. Comparison of techniques for measurement of rumen pH in lactating dairy cows. *J. Dairy Sci.* 87, 59–66.

Eckersall, P.D., Duthie, S., Safi, S., Moffatt, D., Horadagoda, N.U., Doyle, S., Parton, R., Bennett, D., & Fitzpatrick, J.L., 1999. An automated biochemical assay for haptoglobin: prevention of interference from albumin. *Comp. Haematol. Int.* 9, 117–124.

Emery, R.S., & Herdt, T.H., 1991. Lipid nutrition. *Vet. Clin. North Am. Food Anim. Pract.* 7, 341–52.

Enemark, J.M.D., Peters, G., & Jorgensen, R.J., 2003. Continuous Monitoring of Rumen pH - A Case Study With Cattle. *J. Vet. Med. Ser. A* 50, 62–66.

Firkins, J.L., & Yu, Z., 2015. Ruminant Nutrition Symposium: How to use data on the rumen microbiome to improve our understanding of ruminant nutrition1,2. *J. Anim. Sci.* 93, 1450–1470.

Gao, X., & Oba, M., 2016. Effect of increasing dietary nonfiber carbohydrate with starch, sucrose, or lactose on rumen fermentation and productivity of lactating dairy cows. *J. Dairy Sci.* 99, 291–300.

Garrett, E.F., Pereira, M.N., Nordlund, K.V., Armentano, L.E., Goodger, W.J., & Oetzel, G.R., 1999. Diagnostic methods for the detection of subacute ruminal acidosis in dairy cows. *J. Dairy Sci.* 82, 1170–1178.

Gozho, G.N., Krause, D.O., & Plaizier, J.C., 2007. Ruminal lipopolysaccharide concentration and inflammatory response during grain-induced subacute ruminal acidosis in dairy cows. *J. Dairy Sci.* 90, 856–866.

Gozho, G.N., Plaizier, J.C., Krause, D.O., Kennedy, A.D., & Wittenberg, K.M., 2005. Subacute ruminal acidosis induces ruminal lipopolysaccharide endotoxin release and triggers an inflammatory response. *J. Dairy Sci.* 88, 1399–403.

Graham, C., & Simmons, N.L., 2005. Functional organization of the bovine rumen epithelium. *Am. J. Physiol. Integr. Comp. Physiol.* 288, R173–R181.

Gregory, P.C., 1987. Inhibition of reticulo-ruminal motility by volatile fatty acids and lactic

acid in sheep. *J. Physiol.* 382, 355–71.

Grovum, W.L., 1981. Factors affecting the voluntary intake of food by sheep. 3. The effect of intravenous infusions of gastrin, cholecystokinin and secretin on motility of the reticulo-rumen and intake. *Br. J. Nutr.* 45, 183–201.

Gruninger, R.J., Ribeiro, G.O., Cameron, A., & McAllister, T.A., 2019. Invited review: Application of meta-omics to understand the dynamic nature of the rumen microbiome and how it responds to diet in ruminants. *animal* 13, 1843–1854.

Guo, J., Chang, G., Zhang, K., Xu, L., Jin, D., Bilal, M.S., & Shen, X., 2017. Rumen-derived lipopolysaccharide provoked inflammatory injury in the liver of dairy cows fed a high-concentrate diet. *Oncotarget* 8, 46769–46780.

Habel, R.E., 1956. A study of the innervation of the ruminant stomach. *Cornell Vet.* 46, 555–633.

Hamilton, A.W., Davison, C., Tachtatzis, C., Andonovic, I., Michie, C., Ferguson, H.J., Somerville, L., & Jonsson, N.N., 2019. Identification of the rumination in cattle using support vector machines with motion-sensitive bolus sensors. *Sensors* 19, 1165.

Harding, R., & Leek, B.F., 1972. The effects of peripheral and central nervous influences on gastric centre neuronal activity in sheep. *J. Physiol.* 225, 309–338.

Herskin, M.S., Munksgaard, L., & Ladewig, J., 2004. Effects of acute stressors on nociception, adrenocortical responses and behavior of dairy cows. *Physiol. Behav.* 83, 411–20.

Hoover, W.H., & Miller, T.K., 1991. Rumen digestive physiology and microbial ecology. *Vet. Clin. North Am. Food Anim. Pract.* 7, 311–25.

Horadagoda, N.U., Knox, K.M., Gibbs, H.A., Reid, S.W., Horadagoda, A., Edwards, S.E., & Eckersall, P.D., 1999. Acute phase proteins in cattle: discrimination between acute and chronic inflammation. *Vet. Rec.* 144, 437–41.

Huang, Y., Marden, J.P., Julien, C., & Bayourthe, C., 2018. Redox potential: An intrinsic parameter of the rumen environment. *J. Anim. Physiol. Anim. Nutr. (Berl).* 102, 393–402.

Humer, E., Aschenbach, J.R., Neubauer, V., Kröger, I., Khiaosa-ard, R., Baumgartner, W., & Zebeli, Q., 2018a. Signals for identifying cows at risk of subacute ruminal acidosis in dairy veterinary practice. *J. Anim. Physiol. Anim. Nutr. (Berl).* 102, 380–392.

Humer, E., Khol-Parisini, A., Gruber, L., Gasteiner, J., Abdel-Raheem, S.M., & Zebeli, Q., 2015. Long-term reticuloruminal pH dynamics and markers of liver health in early-lactating cows of various parities fed diets differing in grain processing. *J. Dairy Sci.* 98, 6433–48.

Humer, E., Petri, R.M., Aschenbach, J.R., Bradford, B.J., Penner, G.B., Tafaj, M., Südekum, K.-H., & Zebeli, Q., 2018b. Invited review: practical feeding management recommendations to mitigate the risk of subacute ruminal acidosis in dairy cattle. *J. Dairy Sci.* 101, 872–888.

Hungate, R.E., 1966. The rumen and its microbes. Academic Press, New York.

Hut, P.R., Mulder, A., van den Broek, J., Hulsen, J.H.J.L., Hooijer, G.A., Stassen, E.N., van Eerdenburg, F.J.C.M., & Nielen, M., 2019. Sensor based eating time variables of dairy cows in the transition period related to the time to first service. *Prev. Vet. Med.* 169, 104694.

Jenkins, T.C., & Fotouhi, N., 1990. Effects of lecithin and corn oil on site of digestion, ruminal fermentation and microbial protein synthesis in sheep. *J. Anim. Sci.* 68, 460.

Jenkins, T.C., & Jenny, B.F., 1989. Effect of hydrogenated fat on feed intake, nutrient digestion, and lactation performance of dairy cows. *J. Dairy Sci.* 72, 2316–24.

Jonsson, N.N., Kleen, J.L., Wallace, R.J., Andonovic, I., Michie, C., Farish, M., Mitchell, M., Duthie, C.-A., Jensen, D.B., & Denwood, M.J., 2019. Evaluation of reticuloruminal pH measurements from individual cattle: sampling strategies for the assessment of herd status. *Vet. J.* 243, 26–32.

Khafipour, E., Krause, D.O., & Plaizier, J.C., 2009a. A grain-based subacute ruminal acidosis challenge causes translocation of lipopolysaccharide and triggers inflammation. *J. Dairy Sci.* 92, 1060–1070.

Khafipour, E., Krause, D.O., & Plaizier, J.C., 2009b. Alfalfa pellet-induced subacute ruminal acidosis in dairy cows increases bacterial endotoxin in the rumen without causing inflammation. *J. Dairy Sci.* 92, 1712–24.

Khafipour, E., Li, S., Plaizier, J.C., & Krause, D.O., 2009. Rumen microbiome composition determined using two nutritional models of subacute ruminal acidosis. *Appl. Environ. Microbiol.* 75, 7115–24.

Kimura, A., Sato, S., Kato, T., Ikuta, K., Yamagishi, N., Okada, K., Mizuguchi, H., & Ito, K., 2012. Relationship between pH and Temperature in the Ruminal Fluid of Cows, Based on a Radio-Transmission pH-Measurement System. *J. Vet. Med. Sci.* 74, 1023–1028.

Kitkas, G., Valergakis, G., Karatzias, H., & Panousis, N., 2013. Subacute ruminal acidosis: prevalence and risk factors in Greek dairy herds. *Iran. J. Vet. Res.* 14, 183–189.

Kleen, J.L., Hooijer, G.A., Rehage, J., & Noordhuizen, J.P.T.M., 2009. Subacute ruminal acidosis in Dutch dairy herds. *Vet. Rec.* 164, 681–3.

Kleen, J.L., Upgang, L., & Rehage, J., 2013. Prevalence and consequences of subacute

ruminal acidosis in German dairy herds. *Acta Vet. Scand.* 55, 48.

Klevenhusen, F., Pourazad, P., Wetzels, S.U., Qumar, M., Khol-Parisini, A., & Zebeli, Q., 2014. Technical note: Evaluation of a real-time wireless pH measurement system relative to intraruminal differences of digesta in dairy cattle1,2. *J. Anim. Sci.* 92, 5635–5639.

Krajcarski-Hunt, H., Plaizier, J.C., Walton, J.P., Spratt, R., & McBride, B.W., 2002. Short communication: Effect of subacute ruminal acidosis on in situ fiber digestion in lactating dairy cows. *J. Dairy Sci.* 85, 570–3.

Krause, K.M., & Oetzel, G.R., 2006. Understanding and preventing subacute ruminal acidosis in dairy herds: A review. *Anim. Feed Sci. Technol.* 126, 215–236.

Kunst, A., Drager, B., & Ziegenhorn, J., 1984. UV methods with hexokinase and glucose - 6-phosphate dehydrogenase, in: Bergmeyer, H. (Ed.), Method of Enzymatic Analysis. Verlag Chemie, Vol 6, Weinheim, Germany, pp. 163–172.

Lanzas, C., Sniffen, C., Seo, S., Tedeschi, L., & Fox, D., 2007. A revised CNCPS feed carbohydrate fractionation scheme for formulating rations for ruminants. *Anim. Feed Sci. Technol.* 136, 167–190.

LeBlanc, S., 2010. Monitoring metabolic health of dairy cattle in the transition period. *J. Reprod. Dev.* 56 Suppl, S29-35.

Leek, B.F., 1983. Clinical diseases of the rumen: a physiologist's view. *Vet. Rec.* 113, 10–4.

Leek, B.F., 1969. Reticulo-ruminal function and dysfunction. *Vet. Rec.* 84, 238–43.

Li, S., Khafipour, E., Krause, D.O., Kroeker, A., Rodriguez-Lecompte, J.C., Gozho, G.N., & Plaizier, J.C., 2012. Effects of subacute ruminal acidosis challenges on fermentation and endotoxins in the rumen and hindgut of dairy cows. *J. Dairy Sci.* 95, 294–303.

Lochmiller, R.L., & Deerenberg, C., 2000. Trade-offs in evolutionary immunology: just what is the cost of immunity? *Oikos* 88, 87–98.

Martel, C.A., Titgemeyer, E.C., Mamedova, L.K., & Bradford, B.J., 2011. Dietary molasses increases ruminal pH and enhances ruminal biohydrogenation during milk fat depression. *J. Dairy Sci.* 94, 3995–4004.

Matthews, C., Crispie, F., Lewis, E., Reid, M., O'Toole, P.W., & Cotter, P.D., 2019. The rumen microbiome: a crucial consideration when optimising milk and meat production and nitrogen utilisation efficiency. *Gut Microbes* 10, 115.

Mojtahedi, M., & Danesh Mesgaran, M., 2011. Effects of the inclusion of dried molassed sugar beet pulp in a low-forage diet on the digestive process and blood biochemical parameters of Holstein steers. *Livest. Sci.* 141, 95–103.

Morgante, M., Stelletta, C., Berzaghi, P., Gianesella, M., & Andrighetto, I., 2007. Subacute rumen acidosis in lactating cows: an investigation in intensive Italian dairy herds. *J. Anim. Physiol. Anim. Nutr. (Berl).* 91, 226–234.

Morgavi, D.P., Kelly, W.J., Janssen, P.H., & Attwood, G.T., 2013. Rumen microbial (meta)genomics and its application to ruminant production. *animal* 7, 184–201.

Mu, Y., Lin, X., Wang, Z., Hou, Q., Wang, Y., & Hu, Z., 2019. High-production dairy cattle exhibit different rumen and fecal bacterial community and rumen metabolite profile than low-production cattle. *Microbiologyopen* 8, e00673.

Nagaraja, T.G., & Lechtenberg, K.F., 2007. Liver Abscesses in Feedlot Cattle. *Vet. Clin. North Am. Food Anim. Pract.* 23, 351–369.

Nelson, C.D., Reinhardt, T.A., Lippolis, J.D., Sacco, R.E., & Nonnecke, B.J., 2012. Vitamin D Signaling in the Bovine Immune System: A Model for Understanding Human Vitamin D Requirements. *Nutrients* 4, 181–196.

Neubauer, V., Humer, E., Kröger, I., Braid, T., Wagner, M., & Zebeli, Q., 2018. Differences between pH of indwelling sensors and the pH of fluid and solid phase in the rumen of dairy cows fed varying concentrate levels. *J. Anim. Physiol. Anim. Nutr. (Berl).* 102, 343–349.

Nocek, J.E., 1997. Bovine Acidosis: Implications on Laminitis. *J. Dairy Sci.* 80, 1005–1028.

Nogami, H., Arai, S., Okada, H., Zhan, L., & Itoh, T., 2017. Minimized Bolus-Type Wireless Sensor Node with a Built-In Three-Axis Acceleration Meter for Monitoring a Cow's Rumen Conditions. *Sensors* 17, 687.

Nordlund, K.V., & Garrett, E.F., 1994. Rumenocentesis: A technique for the diagnosis of subacute rumen acidosis in dairy herds. *Practitioner* 109–112.

Nordlund, K.V., Garrett, E.F., & Oetzel, G.R., 1995. Herd-based rumenocentesis: a clinical approach to the diagnosis of subacute rumen acidosis. *Compend. Contin. Educ. Pract. Vet. Compend. Contin. Educ. Pract. Vet.*

O'Grady, L., Doherty, M.L., & Mulligan, F.J., 2008. Subacute ruminal acidosis (SARA) in grazing Irish dairy cows. *Vet. J.* 176, 44–9.

Oba, M., 2011. Review: effects of feeding sugars on productivity of lactating dairy cows. *Can. J. Anim. Sci.* 91, 37–46.

Oetzel, G.R., 2017. Diagnosis and Management of Subacute Ruminal Acidosis in Dairy Herds. *Vet. Clin. North Am. Food Anim. Pract.* 33, 463–480.

Onaga, T., 2007. Effect of intravenous infusion of proglumide on ruminal motility in conscious sheep (Ovis aries). *Vet. Res. Commun.* 31, 1021–1036.

Owens, F.N., Secrist, D.S., Hill, W.J., & Gill, D.R., 1998. Acidosis in cattle: a review. *J.*

Anim. Sci. 76, 275.

Penner, G.B., & Oba, M., 2009. Increasing dietary sugar concentration may improve dry matter intake, ruminal fermentation, and productivity of dairy cows in the postpartum phase of the transition period. *J. Dairy Sci.* 92, 3341–53.

Phillips, C.J.C., 1993. Cattle Behaviour, First. ed. Farming Press, Ipswich, UK.

Plaizier, J.C., Danesh Mesgaran, M., Derakhshani, H., Golder, H., Khafipour, E., Kleen, J.L., Lean, I., Loor, J., Penner, G., & Zebeli, Q., 2018. Review: enhancing gastrointestinal health in dairy cows. *Animal* 12, s399–s418.

Plaizier, J.C., Khafipour, E., Li, S., Gozho, G.N., & Krause, D.O., 2012. Subacute ruminal acidosis (SARA), endotoxins and health consequences. *Anim. Feed Sci. Technol.* 172, 9–21.

Plaizier, J.C., Krause, D.O., Gozho, G.N., & McBride, B.W., 2008. Subacute ruminal acidosis in dairy cows: The physiological causes, incidence and consequences. *Vet. J.* 176, 21–31.

Plaizier, J.C., Li, S., Tun, H.M., & Khafipour, E., 2017. Nutritional models of experimentally-induced subacute ruminal acidosis (SARA) differ in their impact on rumen and hindgut bacterial communities in dairy cows. *Front. Microbiol.* 7, 2128.

Qin, C., Bu, D., Sun, P., Zhao, X., Zhang, P., & Wang, J., 2017. Effects of corn straw or mixed forage diet on rumen fermentation parameters of lactating cows using a wireless data logger. *Anim. Sci. J.* 88, 259–266.

R Core Team, 2018. R: a language and environment for statistical computing.

Richter, M., Křížová, L., & Třináctý, J., 2010. The effect of individuality of animal on diurnal pattern of pH and redox potential in the rumen of dry cows. *Czech J. Anim. Sci.* 55, 401–407.

Russell, J.R., & Hino, T., 1985. Regulation of lactate production in Streptococcus bovis: A spiraling effect that contributes to rumen acidosis. *J. Dairy Sci.* 68, 1712–21.

Rutten, C.J., Velthuis, A.G.J., Steeneveld, W., & Hogeveen, H., 2013. Invited review: Sensors to support health management on dairy farms. *J. Dairy Sci.* 96, 1928–1952.

Saleem, F., Ametaj, B.N., Bouatra, S., Mandal, R., Zebeli, Q., Dunn, S.M., & Wishart, D.S., 2012. A metabolomics approach to uncover the effects of grain diets on rumen health in dairy cows. *J. Dairy Sci.* 95, 6606–6623.

Sanford, J., 1963. Formation of histamine in ruminal fluid. *Nature*.

Schirmann, K., von Keyserlingk, M.A.G., Weary, D.M., Veira, D.M., & Heuwieser, W., 2009. Technical note: Validation of a system for monitoring rumination in dairy cows. *J. Dairy Sci.* 92, 6052–6055.

Sellers, A.F., & Stevens, C.E., 1966. Motor functions of the ruminant forestomach. *Physiol. Rev.* 46, 634–661.

Sjaastad, O. V., Hove, K., & Sand, O., 2003. The digestive system, in: Physiology of Domestic Animals. Scandinavian Veterinary Press, Oslo, pp. 490–564.

smaXtec, A.C., 2019. smaXtec pH plus bolus [WWW Document].

Stangaferro, M.L., Wijma, R., Caixeta, L.S., Al-Abri, M.A., & Giordano, J.O., 2016a. Use of rumination and activity monitoring for the identification of dairy cows with health disorders: Part I. Metabolic and digestive disorders. *J. Dairy Sci.* 99, 7395–7410.

Stangaferro, M.L., Wijma, R., Caixeta, L.S., Al-Abri, M.A., & Giordano, J.O., 2016b. Use of rumination and activity monitoring for the identification of dairy cows with health disorders: Part II. Mastitis. *J. Dairy Sci.* 99, 7411–7421.

Steele, M.A., AlZahal, O., Hook, S.E., Croom, J., & McBride, B.W., 2009. Ruminal acidosis and the rapid onset of ruminal parakeratosis in a mature dairy cow: a case report. *Acta Vet. Scand.* 51, 39.

Steele, M.A., Croom, J., Kahler, M., AlZahal, O., Hook, S.E., Plaizier, K., & McBride, B.W., 2011. Bovine rumen epithelium undergoes rapid structural adaptations during grain-induced subacute ruminal acidosis. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 300, R1515-23.

Stefańska, B., Nowak, W., Komisarek, J., Taciak, M., Barszcz, M., & Skomiał, J., 2017. Prevalence and consequence of subacute ruminal acidosis in Polish dairy herds. *J. Anim. Physiol. Anim. Nutr. (Berl).* 101, 694–702.

Steiner, S., & Wittek, T., 2017. The occurrence of sub-acute ruminal acidosis in lower Austrian dairy herds. *Wiener tierärztliche Monatsschrift* 104, 289–296.

Sun, X., Yuan, X., Chen, L., Wang, T., Wang, Z., Sun, G., Li, Xiaobing, Li, Xinwei, & Liu, G., 2017. Histamine Induces Bovine Rumen Epithelial Cell Inflammatory Response via NF-κB Pathway. *Cell. Physiol. Biochem.* 42, 1109–1119.

Timsit, E., Assié, S., Quiniou, R., Seegers, H., & Bareille, N., 2011. Early detection of bovine respiratory disease in young bulls using reticulo-rumen temperature boluses. *Vet. J.* 190, 136–142.

Ulich, T.R., del Castillo, J., Guo, K., & Souza, L., 1989. The hematologic effects of chronic administration of the monokines tumor necrosis factor, interleukin-1, and granulocyte-colony stimulating factor on bone marrow and circulation. *Am. J. Pathol.* 134, 149–59.

Underwood, W.J., 1992. rumen lactic acidosis. Part I. Epidemiology and Pathophysiology. *Compend. Contin. Educ. Pract. Vet.* 14, 1127–1133.

Van Erp-Van der Kooij, E., Van De Brug, M., & Roelofs, J.B., 2016. Validation of Nedap

Smarttag Leg and Neck to assess behavioural activity level in dairy cattle. *Procceding Precis. Dairy Farming* 321–326.

Van Soest, P.J., 1994. Microbes in the gut, in: Nutritional Ecology of the Ruminant. Cornell University Press, pp. 253–280.

Wang, A., Gu, Z., Heid, B., Akers, R.M., & Jiang, H., 2009. Identification and characterization of the bovine G protein-coupled receptor GPR41 and GPR43 genes. *J. Dairy Sci.* 92, 2696–705.

Wang, D.S., Zhang, R.Y., Zhu, W.Y., & Mao, S.Y., 2013. Effects of subacute ruminal acidosis challenges on fermentation and biogenic amines in the rumen of dairy cows. *Livest. Sci.* 155, 262–272.

Youm, Y.-H., Nguyen, K.Y., Grant, R.W., Goldberg, E.L., Bodogai, M., Kim, D., D'Agostino, D., Planavsky, N., Lupfer, C., Kanneganti, T.D., Kang, S., Horvath, T.L., Fahmy, T.M., Crawford, P.A., Biragyn, A., Alnemri, E., & Dixit, V.D., 2015. The ketone metabolite β-hydroxybutyrate blocks NLRP3 inflammasome-mediated inflammatory disease. *Nat. Med.* 21, 263–9.

Zebeli, Q., & Ametaj, B.N., 2009. Relationships between rumen lipopolysaccharide and mediators of inflammatory response with milk fat production and efficiency in dairy cows. *J. Dairy Sci.* 92, 3800–3809.

Zebeli, Q., Dijkstra, J., Tafaj, M., Steingass, H., Ametaj, B.N., & Drochner, W., 2008. Modeling the adequacy of dietary fiber in dairy cows based on the responses of ruminal pH and milk fat production to composition of the diet. *J. Dairy Sci.* 91, 2046–66.

Zhang, N., Ahsan, M.H., Purchio, A.F., & West, D.B., 2005. Serum amyloid A-luciferase transgenic mice: response to sepsis, acute arthritis, and contact hypersensitivity and the effects of proteasome inhibition. *J. Immunol.* 174, 8125–34.